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A Summary of Current Program and
Preliminary Report of Progress

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DAIRY RESEARCH

of the

United States Department of Agriculture
and related work of the
State Agricultural Experiment Stations

This progress report is primarily a research tool for use of scientists and administrators in program coordination, development, and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of research progress include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members, and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of U.S.D.A. and cooperative research issued during the past year. Current agricultural research findings are also published in the monthly U.S.D.A. publications, Agricultural Research and The Farm Index.

UNITED STATES DEPARTMENT OF AGRICULTURE
Washington, D. C. 20250

December 1, 1964

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CURRENT SERIAL RECORDS

ADVISORY COMMITTEES

The research program of the Department of Agriculture is reviewed annually by the following advisory committees:

1. Farm Resources and Facilities Research
2. Utilization Research and Development
3. Human Nutrition and Consumer Use Research
4. Marketing Research
5. Agricultural Economics Research
6. Forestry Research
7. Animal and Animal Products Research
8. Cotton Research
9. Grain and Forage Crops Research
10. Horticultural Crops Research
11. Oilseed, Peanut and Sugar Crops Research
12. Plant Science and Entomology
13. Tobacco Research

ORGANIZATIONAL UNIT PROGRESS REPORTS

The source materials used by the advisory committees are of two types. First, there are Organizational Unit Reports that cover the work of the Divisions or Services listed below. The number prefixes refer to advisory committees listed above that review all of the work of the respective Divisions or Services.

Agricultural Research Service, (ARS)

- 1 - Agricultural Engineering
- 1 - Soil & Water Conservation
- 2 - Utilization -- Eastern
- 2 - Utilization -- Northern
- 2 - Utilization -- Southern
- 2 - Utilization -- Western
- 3 - Human Nutrition
- 3 - Clothing and Housing
- 3 - Consumer & Food Economics
- 4 - Market Quality
- 4 - Transportation & Facilities
- 7 - Animal Husbandry
- 7 - Animal Disease & Parasite
- 12 - Crops
- 12 - Entomology

Economic Research Service, ERS)

- 4,5 - Marketing Economics
- 4 - Farm Production Economics
- 5 - Resource Development Economics
- 5 - Economic & Statistical Analysis
- 5 - Foreign Development & Trade Analysis
- 5 - Foreign Analysis

Other Services

- 4,5 - Farmer Cooperative Service (FCS)
- 4,5 - Statistical Reporting Service (SRS)
- 6 - Forest Service (FS)

SUBJECT MATTER PROGRESS REPORTS

The other type of report brings together the U.S.D.A. program and progress for the following commodities or subjects:

- | | |
|--|------------------------------------|
| 3 - Rural Dwellings | 8 - Cotton and Cottonseed |
| 6 - Forestry (other than Forest Service) | 9 - Grain and Forage Crops |
| 7 - Beef Cattle | 10 - Citrus and Subtropical Fruit |
| 7 - Dairy | 10 - Deciduous Fruit and Tree Nut |
| 7 - Poultry | 10 - Potato |
| 7 - Sheep and Wool | 10 - Vegetable |
| 7 - Swine | 10 - Florist, Nursery & Shade Tree |
| 7 - Cross Specie and Miscellaneous | 11 - Oilseeds and Peanut |
| Animal Research | 11 - Sugar |
| | 13 - Tobacco |

A copy of any of the reports may be requested from Max Hinds, Executive Secretary, Animal and Animal Products Research Advisory Committee, Research Program Development and Evaluation Staff, U. S. Department of Agriculture, Washington, D. C.

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See also Cross Specie and Miscellaneous Animal Research report for:

- Animal Biology: basic research on genetics, nutrition and physiology.
- Engineering work applicable to farmstead planning including chore time standards, model layouts, and water supply.
- Miscellaneous Diseases and their aspects such as serum, bloat, laboratory diagnosis, toxicology and pathology related to insecticides, biochemical effects of agricultural chemicals, poisoning by plants, and related programs.
- Parasites: collection, index catalogue, removal of.

INTRODUCTION

This report on dairy research covers work directly related to the production, processing, distribution, and consumption of milk and its products. The information has been assembled from the organizational unit reports of the several divisions. This report does not include extensive cross-commodity work, much of which is basic in character, which contributes to the solution of not only dairy problems but also to the problems of other commodities. Progress on cross-commodity work is found in the reports of the several divisions such as Soil and Water Conservation, Human Nutrition, Transportation and Facilities, Farm Production Economics, Foreign Development and Trade Analysis, and Cross-Species and Miscellaneous Animal Research.

This report is devoted to the 21 "problem areas" shown in the table of contents. For each area there is a statement of (1) the Problem, (2) the USDA and Cooperative Program, (3) Program of State Experiment Stations, (4) a summary of Progress during the past year on USDA and cooperative work, and (5) a list of Publications resulting from USDA and cooperative work.

Dairy research can be divided into three major categories, i.e., that supported by (1) Federal funds appropriated to the research agencies of the United States Department of Agriculture, (2) Federal and State funds appropriated to the 53 State Agricultural Experiment Stations, and (3) private funds allotted, largely by the dairy industry, to research carried on in private laboratories or to support of State Station or USDA work. For all three categories it is estimated that about 2,100 scientists are engaged in research dealing specifically with the production, processing, distribution, and consumption of dairy animals, milk, and its products. Support of their work involves an annual expenditure of between 50 and 55 million dollars. This amounts to about 0.8 percent of the cash farm receipts from the sale of dairy animals for slaughter and dairy products and about 0.4 percent of the retail value of the same items. Of the 2,100 scientists engaged in dairy research, approximately 14% are employed by the Department of Agriculture, 21% by the State Experiment Stations, and 65% by other universities, foundations, and private industry.

Research by USDA

Farm research pertaining to dairy is conducted in the Agricultural Research Service divisions of Agricultural Engineering, Animal Disease and Parasite, Animal Husbandry, and Entomology. The work comprises investigations of breeding, physiology, nutrition, diseases and parasites, housing and management of dairy animals, and sanitary handling and storing of milk until it leaves the farm. The work involves 186 professional man-years of scientific effort which includes research on cattle diseases and parasites that is applicable to beef cattle, also.

Nutrition, consumer, and utilization use research pertaining to dairy is conducted in the Agricultural Research Service divisions of Human Nutrition, Consumer and Food Economics, and Eastern Utilization. The work comprises investigations of composition and nutritive value; physiological availability of nutrients and their effects; new and improved methods of preparation, preservation, and care in homes, eating establishments and institutions; and with the processing phase involving pasteurization, bottling, separation of cream and skim milk; manufacture into products such as butter, powder, cheese, concentrated forms, ice cream, and numerous specialty items. Also, it is concerned with improved equipment and processes. The work in these divisions involves 99 professional man-years of scientific effort.

Marketing and economic research pertaining to dairy is carried on within four Services: Agricultural Research Service, Economic Research Service, Farmer Cooperative Service, and Statistical Reporting Service. The work comprises (1) physical and biological aspects of assembly, packaging, transporting, storing and distribution; (2) economic aspects of marketing costs, margins and efficiency, market potential, supply and demand, and situation and outlook; (3) cooperative marketing, and (4) consumer acceptance studies. The divisions in which the work is conducted are: Market Quality, ARS; Transportation and Facilities, ARS; Marketing Economics, ERS; Economic and Statistical Analysis, ERS; Marketing Division, FCS; Standards and Research, SRS. The scientific effort involved by these divisions amounts to 24 professional man-years.

Interrelationships among Department, State and Private Research

A large part of the Department's research is cooperative with State Experiment Stations. Many Department employees are located at State Stations and use laboratory and office space close to or furnished by the Station. Cooperative work is jointly planned, frequently with the participation of representatives of the producers or industry affected. The nature of cooperation varies with each study. It is developed so as to fully utilize the personnel and other resources of the cooperators which frequently includes resources contributed by the interested producers or industry.

Including both cooperative and State Station projects dairy research is carried on by 52 State Experiment Stations. The types of work to which the largest amount of effort is devoted include nutrition and management, diseases, breeding, physiology, utilization and marketing. There is regular exchange of information between Station and Department scientists to assure that the programs complement each other and to eliminate unnecessary duplication.

Privately supported dairy research emphasizes the solution of scientific production, processing, and marketing problems. Much of it utilizes the results of basic work done by State Station and Department scientists.

A number of companies make application of basic research developed through public research on products intended for prevention, control, or treatment of diseases, parasites and insects; however, final evaluation is often done cooperatively with public agencies. Most of the identification and classification of insects, diseases and parasites is done by public institutions but the information is available for use by individuals and firms.

About 1/3 of the industry research effort in the dairy industry is in the utilization field. Very little of the work is basic and where it is, the results are usually patented. In applied research the major activities are in cost saving, container testing and low calorie product formulation. Public research was used to develop the procedure for removal of strontium-90 from milk.

The contributions of dairy producers and industry to the work of the State Stations and the Department have been an important factor in the success of their research programs. Producers offer herds and facilities for testing products and practices used in production. Likewise, processors and retailers offer facilities and products for use by public research agencies. Many problems in the economics of marketing cannot be transferred to a laboratory, experimental plot, or other simulated situation. The results of economic research conducted cooperatively is of great value to industry, especially in cases where public research can provide comparison and analysis. Even large firms that have a research staff do not have access to the plants and records of competitors.

Examples of Recent Research Accomplishments by USDA and Cooperating Scientists

Importance of dairy type. Although many studies have reported that the relationship between dairy type and milk production is low, breeders still have placed great emphasis on type because they thought it contributed to length of productive life. Beltsville workers have shown, however, that most dairy cows leave herds for reasons not related to present standards of dairy type. These reasons were largely environmental. Therefore, efforts to improve length of life or production through selection for high dairy type scores are ineffective. The main value of high type scores is related to the sale value of the animals among registered breeders.

Bovine Vibriosis. Researchers at the National Animal Disease Laboratory reported that all heifers bred to an infected bull became infected at the first service. Only 2 of 12 infected heifers became pregnant as compared to 6 pregnancies in the 7 noninfected control heifers. The presence of a moderate inflammatory process in the uteri of the infected heifers suggested the underlying cause for the lack of conception.

Reproductive Diseases. Much progress is being made in determining causes of reproductive diseases in cattle. California, Colorado and Ohio workers recently have discovered that outbreaks of abortion can be caused by infectious bovine rhinotracheitis - a virus disease which previously was thought to affect only the respiratory tract of cattle. Connecticut research has found some cases of bovine sterility to be due to infection with Mycoplasma - an organism with bacterial and viral-like characteristics. Severe outbreaks of mastitis also were traced by these workers to a similar Mycoplasma agent.

Ventilation of Livestock Buildings. Research in cooperation with State Experiment Stations has obtained much needed basic data on the heat and moisture given off by cattle, hogs, and poultry, and on the influence of building environment on production and feed consumption. The heat and moisture dissipation data are considered basic design data for ventilation systems of poultry, dairy, and swine buildings. They appear in design handbooks including the 1964 Guide and Data Book of the American Society of Heating, Refrigeration, Ventilating, and Air Conditioning Engineers, and are used by makers of ventilating equipment, prefabricated buildings and package buildings as well as by specialists advising farmers on their own construction. Building improvements resulting from the above research have contributed to the substantial rise in efficiency of livestock production that has occurred during the past decade.

New Method for Making Dried Whey in Commercial Use. The foam-spray method for drying cheese whey and other dairy products developed by the Eastern Utilization Research and Development Division scientists is now being used by several large manufacturers of cottage cheese to make dry whey for food use. One large company alone can produce 3 million pounds of dry whey annually. The novel feature of the new process is injection of air into concentrated whey immediately before it enters the atomizer in the spray-drying chamber. The resulting dry product is free-flowing and disperses readily in contrast to dry whey produced by conventional spray-drying methods, which tends to be sticky and lumpy and does not reconstitute readily. Industry has found the new type drying equipment relatively inexpensive to install and operate. The entire cottage cheese industry has a potential to

produce nearly a billion pounds of dry whey annually. Since most of the cottage cheese whey production has been disposed as a waste material because of lack of a profitable outlet for it, this development represents a large potential for increased income to the dairy industry, and at the same time it tends to alleviate the problem of stream pollution by dairy wastes.

Improved Low-Fat Ripened Cheese Developed. A new method for making low-fat ripened cheese, a product much desired by diet-conscious consumers, has been developed as a result of research of the Eastern Utilization Research and Development Division, ARS. Heretofore, skim milk cheese of quality suitable for direct consumption has not been available. While some cheese of this type is being produced by conventional methods, it is tough and lacks flavor and is used mostly for processing with other foods. The cheese produced by the new method is relatively soft, mellow, and mild flavored. While somewhat resembling Cheddar cheese, it has only about 5% butterfat in contrast to 31% for Cheddar and contains larger amounts of nonfat milk solids and moisture than Cheddar. The striking improvement in body, texture, and flavor has been accomplished through addition of selected monoglycerides to the skim milk and careful control of operating conditions during processing and curing of the cheese. The "new" cheese should satisfy a sizable consumer demand and provide an appreciable outlet for nonfat milk solids.

Improving Pooling and Pricing Methods of Marketing Cooperatives. Study of marketing methods of dairy cooperatives showed that pooling is conducted in one form or another by all these associations. Over 600,000 dairy farmers participate in these pools. The specific procedures vary greatly. Emphasis in this study is on designing and selecting milk pooling methods that will be effective and equitable to all producers in the new market environment.

I. FARM RESEARCH

DAIRY CATTLE - BREEDING

Animal Husbandry Research Division, ARS

Problem. Dairymen need information on genetic methods for increasing the efficiency of milk production and modifying milk composition, as well as other economic traits, in order to reduce unit costs and meet the future market demands. Precise information is needed on the relative importance of performance traits, the nature of their inheritance and their response to selection and specific systems of mating. Advanced genetic methods, such as those utilizing heterosis and specific and general combining ability, need to be evaluated as procedures for more rapid improvement of milk production or other important traits.

USDA AND COOPERATIVE PROGRAM

This is a continuing program conducted by geneticists on basic and applied studies of the inheritance of the dairy cow, including experiments designed for evaluating the application of advanced genetic concepts to dairy cattle improvement. The work is in progress at Beltsville, Maryland, and cooperatively with 14 State experiment stations and laboratories in nine foreign countries. Several of the studies contribute to the North Central and Southern regional dairy cattle breeding projects. Cooperation is also carried out with the National Association of Artificial Breeders and with the various dairy cattle breed registry organizations.

The Federal scientific effort devoted to the research in this area totals 18.2 professional man-years. Of this number, 6.0 are devoted to genetics and interrelations of performance traits, 11.0 to selection and systems of breeding, and 1.2 to program leadership.

A grant with the Agricultural Research Center, Tikkurila, Finland, provides for research on the breed differences regarding the antigenic properties of cattle blood, their inheritance in relation to economic characteristics and genetic origin of the breeds. Its duration is for four years, 1961-64, and involves PL-480 funds with a \$61,804 equivalent in Finnish Finmarks.

Another grant with the Division of Investigaciones Agropecuarias, Ministry of Agriculture, Bogota, Colombia, supports work on the evaluation of the native breed, Costeno Con Cuernos, and Holsteins and Brown Swiss when mated and selected for dairy traits under the hot and humid conditions of Northern Colombia. The duration of the grant is for five years, 1962-67, and involves PL-480 funds with a \$246,000 equivalent in Colombian pesos.

Two PL-480 projects (also reported in area 6) S3 AH-7, at Sao Paulo, Brazil, and A7 AH-1, at Izatnagar V.P., India, are in effect and are pertinent to this area.

A contract in the amount of \$20,000 per year is in effect with the Wisconsin Agricultural Experiment Station to study the meat production potential of dairy cattle. Its duration is four years.

PROGRAM OF STATE EXPERIMENT STATIONS

Dairy cattle breeding research is conducted with experiment station and college herds, institutional herds, and privately owned herds; with data available through the DHIA program; and in cooperation with artificial breeding associations in sire proving and similar studies. Areas of investigations include: (1) the estimation of genetic parameters such as heritability, genetic correlations, genetic transmitting ability, and heterosis, and (2) response to programs for genetic improvement. Specific items of study include selection response, milk constituents, crossbreeding, genetic abnormalities, climatic adaptability, blood antigens, milking rate, and efficiency of feed utilization. Much of the research is conducted under two regional projects-- NC-2, Improvement of Dairy Cattle Through Breeding, and S-49, Genetic Methods of Improving Dairy Cattle for the South.

The USDA is cooperating on several research projects with the State agricultural experiment stations and participates actively in the two regional projects.

The total research effort on dairy cattle breeding research by the State agricultural experiment stations is 46.8 professional man years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Genetics and Interrelations of Performance Traits

1. Genetic-environmental interactions in feeding efficiency and consumption. At Lewisburg, Tennessee, a total of 154 first lactation Jersey cows by 11 sires have completed 305 day production records; 77 on roughage only and 77 on roughage plus grain. The average mature equivalent fat corrected milk (ME FCM) yield for the roughage only group was 9,702 pounds as compared to 12,196 pounds for the roughage plus grain group. The roughage only group produced 79.6% as much ME FCM as the roughage plus grain group. The individual sire groups ranged from 68.4 to 83.4%.

Highly significant differences between rations were found for ME milk, ME fat and ME - FCM production. There were also highly significant differences between sires within ration groups for these measurements. The sire X ration interaction was not significant for any of the measures of production. In the no grain group, it was shown that there were differences between sires in the ability of their daughters to consume roughage. This between sire difference in roughage consumption was in the form of both silage and hay. These preliminary results indicate that there is no interaction between sires and rations, but there are differences in the ability of daughters of

different sires to consume forage.

At Utah a total of 112 first lactation Holsteins by 10 sires have finished production records, 52 on forage only and 60 on forage plus grain. Six of the sire groups originated at the Huntley, Montana Station and, 4 sire groups originated at the Utah Station. The average ME FCM yield of the cows fed hay only was 9,584 pounds as compared to 13,664 for the cows fed grain and forage. The range in ME FCM production for the hay only group was from 7,595 to 10,981 pounds, whereas the range for the forage plus grain group was 10,765 to 15,391 pounds. The hay only groups produced 67.2% as much as their half-sibs on forage plus grain. The individual sire groups ranged from 54.8% to 76.8%. An analysis of variance using weighted means indicated significant differences among sires and rations. However, the sire X ration interaction was not significant. The results paralleled those reported from the Jersey data at the Lewisburg, Tennessee station. The most striking difference between the two studies was the higher ME FCM yield of the Jerseys on the all forage ration. The Jerseys on forage only produced 9,702 pounds as compared to 9,584 pounds on the Holsteins at Utah. These results indicate that Jerseys are considerably more efficient than Holsteins in utilizing forage for milk production. (AH g1-4)

2. Ration effects on production efficiency. The research at Beltsville to determine the value of certain feeding regimes in estimating genetic differences in feed efficiency among cows is now completed. The detailed analysis of the data is not completed; however, preliminary results are available on 43 completed lactations from a total of 25 cows fed at a constant rate of feed intake. Their average FCM yield was 13,977 pounds with a feed efficiency index of 1.999. The corresponding standard deviations were 2,022 and 0.27, respectively. Twenty seven cows fed according to production and maintenance requirements have completed 45 lactations. These cows averaged 15,039 pounds of FCM and their efficiency of feed utilization was 1.944. The standard deviation of FCM yield and feed efficiency was 2,626 and 0.194, respectively. The greater yield of 1,062 pounds from the groups fed according to requirements was probably due in part to differences in energy intake. This group consumed an average of 753 therms of additional energy. This would be expected when feed intake is varied with production and maintenance requirement as opposed to feeding at a constant level of energy intake regardless of requirements. The repeatability between successive lactations for FCM yield was 0.378 and 0.623 for the constant fed and requirement fed groups, respectively. The repeatability of feed efficiency was 0.283 and 0.511 for the constant fed and requirement fed group, respectively. The requirement fed group was more persistent and gained more weight during the lactation than the constant fed group. (AH g1-4)

3. Genetic parameters of feed efficiency. A statistical study was made using Beltsville Holstein data to determine heritability of feed efficiency and genetic correlations between efficiency and production. There were 684 lactations by 332 individual cows, which represented 27 sire groups and

included 255 daughter-dam pairs. The means for FCM yield, therms intake and gross feed efficiency after corrections were made for environmental influences were 11,380, 6,520 and 1.74; 13,810, 7,860 and 1.76; 15,360, 8,430 and 1.83; 15,870, 8,760 and 1.81; 16,360, 8,620 and 1.89 for the first, second, third, fourth and fifth lactations, respectively. The increase in gross efficiency as the lactation number increased is probably a reflection of the animal's decreasing need for energy to increase body size. Therefore, a proportionately greater amount of energy is being used for production as the lactation number increases.

The within sire phenotypic correlations for all lactations between FCM yield and feed efficiency, FCM yield and therms intake, and therms intake and feed efficiency were 0.86, 0.74, and 0.31, respectively. These were all significant at the 1% level of probability.

Utilizing the data on 255 daughter-dam pairs over all lactations the genetic correlations between FCM yield and gross efficiency, FCM yield and therms intake, and therms intake and feed efficiency were 0.92, 0.82, and 0.52, respectively. These were all significant at the 1% level of probability. The genetic correlation between FCM yield and feed efficiency of 0.92 indicates that selection for FCM yield would also bring about increased efficiency of feed utilization. The estimates of heritability were 0.52, 0.43 and 0.52 for FCM yield, therms intake and feed efficiency, respectively, on all lactations combined.

Significant sire differences were found for FCM yield, therms intake, and feed efficiency on a first and combined lactation basis.

From these studies it is evident that gross feed efficiency is a heritable trait, and could be increased by selecting directly for it. However, because of the high genetic relationship with production little would be gained by including it in a selection program.

A second objective of this study was to determine if feed efficiency could be estimated from a shorter period of time than a complete lactation. The data in this phase of the study included 525 lactations on 311 cows by 18 sire groups. The feed consumption and FCM production were summarized by 10 day periods for each lactation. The analysis showed a correlation of 0.88 between feed efficiency for the period 121 - 180 days and total lactation. The correlation for the second 100 days of lactation and total lactation was 0.92. These results indicate that feed efficiency can be estimated from relatively short periods of time. (AH gl-4)

4. Relationships between milk yields and rate of milking. Individual quarter milking rate data were collected on 77 Holstein and 42 Jersey cows during second or later lactations. Highly significant differences were found among cows, among quarters of the udder and between morning and evening milking after 15 and 9 hour intervals for total yield per quarter, volume of milk obtained to a minimum rate of .4 lb./min., length of this

portion of the milking and maximum rate of flow. Correlations with total milk were 0.96 for volume of milk to a rate of .4 lb./min., 0.80 for duration of time at this rate and 0.83 for maximum rate. Breed group differences were also significant for the three variables measured. When cows were divided into high and low production groups, differences occurred between them for yield to a rate of .4 lb./min., length of time to .4 lb./min. and maximum rate. When the data were adjusted to a common production level these differences disappeared. No evidence for age effects were found. (AH g1-2)

5. Meat production from beef, dual purpose and dairy steers. This study was initiated in cooperation with the Beef Cattle Research Branch to determine the relative merits of various breeds of cattle and different management systems in the production of meat. The first phase of the second replication of this study is completed. Holsteins, Milking Shorthorns, Jerseys, and Hereford steers were fed during the first 6 months of life either on a high plane of nutrition involving large quantities of whole milk or a low plane of nutrition typical of dairy replacement feeding practice to 6 months of age.

The average daily rate of gain for the high plane group was 2.69, 2.34, 1.95, and 1.79 for the Holsteins, Milking Shorthorns, Herefords, and Jerseys, respectively. Feed efficiency was 0.38, 0.36, 0.32, 0.33 for the Holsteins, Milking Shorthorns, Herefords, and Jerseys, respectively. In the low plane group the average daily rate of gain was 1.28, 1.35, 1.26, and 1.19 for the Holsteins, Milking Shorthorns, Herefords, and Jerseys, respectively. Feed efficiency for this group was 0.38, 0.36, 0.35 and 0.35 for the Holsteins, Milking Shorthorns, Herefords, and Jerseys, respectively. Analysis of variance showed that both breed and treatment differences were significant for average daily rate of gain. However, in feed efficiency only breed differences were significant. As compared to the first replication, the average daily rate of gain increased by 30% for Holsteins, 44% for Milking Shorthorns, 40% for beef breeds, and 46% for Jerseys on the low plane of nutrition. This increase in gain corresponds to an overall average increase in energy intake of 43% between the two replications.

At 180 days of age 13 steers from the high plane group and 12 from the low plane group were slaughtered for carcass evaluations. The average percentage of lean, fat, and bone was 59.1, 19.7, and 21.2 for the high plane group. In the low plane group the corresponding percentages were 62.9, 11.3 and 25.7, respectively. The lean from the 9 - 11 ribs of the high plane steers were significantly more tender as evaluated both by the Warner Bratzler shear and a panel of judges. These results are similar to those obtained in the first replication. (AH d3-6)

6. The genetics of blood antigens in dairy cattle. Blood group specificity is a consequence of the chemical structure of the erythrocyte stroma. Specificity is retained even after ether extraction followed by alkaline aqueous washing. At the Ohio laboratory material produced by this process

was treated with papain. The papain resistant, insoluble residue had only F specificity when tested in inhibition tests. After suitable preparation the soluble portion was passed through a Sephadex G-25 column. Fluant fractions showed two carbohydrate peaks. Material from the first, which coincided with a protein peak, specifically inhibited V blood group factor activity when the substrate was from V-positive cells. No inhibition occurred when V-negative cells were used. Neuraminidase treatment of stroma eliminated F activity but had no effect on V activity. Sialic acid released by the neuraminidase was not effective in inhibiting F reagent even though a concurrent loss of F activity occurred in the sample treated with the enzyme. Two commercial sialic acid derivations, N-acetyl neuraminic acid and N-glycolyneuraminic acid, also proved ineffective in inhibition tests. (AH gl-6)

The PL-480 project in Finland is now in its fourth and final year. The Finnish laboratory has produced 41 blood typing reagents. Two of these, SF-1 and SF-4, are new reagents in the B-system. Another, SF-3, still appears to be unrelated to any of the known bovine blood group systems.

Studies of three cow families failed to indicate any relationship between the inherited polled condition and blood groups. Preliminary studies also failed to indicate relationship between blood groups and congenital hydrops.

Blood group studies of 1,364 dam-daughter combinations (A.I. sires) indicated that 4.33% of the pedigrees were in error on the base of this comparison alone. Sires of dams were in error in 3.01% of the cases so over 7% of the pedigrees were erroneous in the first two generations. These figures compare well with data from other countries.

Analyses of 454 disputed parentage cases (two possible sires) resulted in a solution by blood grouping in 83% of them. When cows were bred to two different bulls during the same estrus period the last sire used was found to be the sire of the calf in about 70% of the cases. This finding is in agreement with the recommendation that cows should be inseminated late in estrus. (PL-480-E8-AH-1)

7. Genetics of milk constituents. The national cooperative effort to obtain data on milk, milk fat, solids-not-fat and protein production of individual cows is continuing. Preliminary analysis of data is in progress at Virginia Polytechnic Institute under the direction of the Interregional Research Committee on Milk Composition. Lactation records numbering 10,361 have been contributed by Northeastern, Southern and North Central Cooperators. The number of lactations by breeds are as follows: Ayrshire 385, Guernsey 1,114, Holstein 5,685, Jersey 2,757, and Brown Swiss 420. The Beltsville contribution to data is 440 lactation records. One preliminary finding is that milk, fat, and solids-not-fat (SNF) yields exhibit a seasonal pattern with means for the periods of November-April and May-October differing significantly. The average milk, fat, and SNF yields were

12,970, 487, and 1,118 pounds for the winter period and 12,050, 454, and 1,045 pounds for the summer period. It is important to note, however, that no seasonal patterns were detected in the percentages of fat and SNF.

The Beltsville analysis is particularly concerned with the use of milk composition information to calculate energy output in the milk. Factors for the calorific value of milk fat, protein and lactose are available from the literature. These are being applied to the data obtained by rapid testing methods to arrive at a production figure for each cow in terms of therms of energy. Since the mineral portion of milk moves very little, it is possible to arrive at lactose percent by subtracting protein percent plus 0.7 (an average for mineral) from SNF percent. Forty-six samples of milk from individual cows were tested by rapid methods for fat, protein and SNF and the therms per pound calculated by the above approximation method. These values were compared with those obtained by bomb calorimetry tests on the same samples of milk. The average therms per pound in these samples was .291. The average difference between values obtained by the two methods, one an approximation and the other a direct tests, was .013 when sign of the difference was not considered. Considering sign the average difference was .006. The correlation between the two methods was .84. Although these are preliminary results it appears that a fair estimate of energy in milk can be made in the manner described above.

In a preliminary attempt to compare results, ten fresh milk samples were sent to laboratories in the North Eastern region. Eight laboratories tested the samples for butterfat by the Babcock method. The average differences from the true value for laboratories ranged from .07 to .20 when sign of difference was not considered. Six laboratories tested the samples for protein using a dye-binding method. The average differences for laboratories between Kjeldahl and dye-binding methods ranged from .048 to .14 (without sign). Five laboratories tested the samples for SNF by gravimetric drying methods and seven tested for SNF using a density method. Six laboratories used the Watson lactometer and one used Golding beads. One of the laboratories used both methods so eight sets of data were available. The average gravimetric value for each sample was taken as the best estimate of the true value and differences for the density methods were calculated as they were for fat and protein. The average differences for laboratories using the Watson method ranged from .105 to .284 (without sign). Corresponding averages for the two laboratories using Golding beads were .278 and .472. Although only a preliminary study, this experiment indicates a need for more work of this kind and focuses attention on certain laboratories that are having difficulty with particular methods. In general, the dye-binding test for protein appears to be more repeatable from laboratory to laboratory than does the density method for SNF.

Although cooperative work with the Eastern Utilization Division is continuing, milk protein typing is now being done at Beltsville for studies of association between these and other genetic polymorphisms and traits of

economic importance. Electrophoretic studies of milk from 165 cows provided further confirmation of the genetic control of variation in α_s and ρ casein and in β -lactoglobulin. The predominant types found continue to be the α_s -B and β -A types of casein. The A and B forms of β -lactoglobulin are found with approximately equal frequency. One use of genetic milk typing which has been demonstrated is in selecting identical twins. Out of ten pairs of twins which were presumed to be identical after blood typing, three were found to be non-identical on the basis of milk protein types. This is probably a much higher percentage of rejections than expected and is probably due to chance in this small sample. However, these findings emphasize how useful these additional genetic markers can be. (AH gl-5)

B. Selection and Systems of Breeding

1. Comparisons of inbreeding and outbreeding. The development of crosses among six inbred lines of Holstein-Friesian cattle and maintenance of controls has continued. Data were analyzed to estimate the effects due to system of mating and age and their interaction. Three of the lines showed similar results in that the interactions between mating system and age on all seven body measurements were not significant. Age effects were very similar between sire lines within each of the measurements. Although body measurements remained relatively constant from one age to another, there existed a great variation among the three sire lines. Inbred calves were generally smaller than non-inbreds. In the other three-sire-lines the results were different. Age trends in growth differed from one mating system to another. The maximum effects of inbreeding on growth seemed to be between 6 and 12 months of age. At older ages, inbreeding accounted for decreasing proportion of the total variation.

Mating system was significant for all body measurements after calving in two lines and most measurements in the other four lines. In general, inbreds were smaller than non-inbreds. In some cases, the outcrosses did not exceed the inbreds of the same sire-line. Lack of significant interaction between mating system and parity were considered as evidence that inbreds did not appear to grow more rapidly and thereby tend to approach the size of the non-inbreds for the period studied.

No interactions were found between mating system and lactation number of various production traits. The change in production with lactation number was the same for all mating systems. Generally, inbred cows produced less milk and fat than the non-inbreds of the same sire line. Inbred cows tended to be older in age at each calving than the outcrosses and line crosses. The differences varied from sire-line to sire-line.

Analysis of 3,076 ovulations covering 728 cow parities revealed the incidence of quiet ovulations to be 23.7%. Both sire-line and season had significant influences on the rate of quiet ovulations. A higher incidence (25.8 vs. 21.5) of quiet ovulations occurred from March 1 to August 31

than from September 1 to February 28. Influence of system of mating was found to be nonsignificant, but its interactions with line and with parity were significant. A highly significant negative correlation between incidence of quiet ovulations and interval since calving in cows or 12 months of age in heifers was noted. A low but highly significant repeatability (0.04) within parity also was found.

A total of 790 parities (728 non-cystic and 62 cystic) covering 3,549 ovulations was analyzed for effects of sire-line, system of mating, parity, cystic condition, and individuality of the cow on the incidence of multiple ovulations. Sire-line had no significant influence. Frequency of multiple ovulations increased in association with the cystic condition (noncystic 4.2, cystic 12.9%) and with increasing parity (2.9, 3.7 and 5.9% for parities one through three). Outbreds had a significant higher incidence of multiple ovulations than inbreds (outbreds 5.2, inbreds 3.2%). A highly significant repeatability (0.07) within a cow also was noted. (AH g2-5)

2. The relative importance of general and specific combining ability in breeding dairy cattle. Inbreeding effects on growth were studied on 2000 females. Four measures of size at ages 3, 6, and 12 months, 3 months after first calving and 3 months after calving at mature age were included in the analysis. Significant negative regressions of inbreeding on heart girth and wither height were found for all ages except maturity. In chest depth all ages were significant. The regressions for body length were significant for 6 months, 12 months and three months after first calving. These results indicate that even at low levels of inbreeding, economic characteristics are depressed.

Over a period of 10 years, 395 pregnant females returned to estrus one or more times during the pregnant period and were bred either naturally or artificially. Heifers appear to return to estrus more frequently in the later stages of pregnancy than older cows. There was a significant decrease in fertility for the animals which were in estrus during pregnancy, for the breeding period following the gestation with estrus. (AH g2-22)

3. The influence of parental relationship on the genetic merit of dairy cows and sires. This research was undertaken to determine the relative merits of line-breeding, outcrossing and crossbreeding using progeny tested bulls of high merit as service sires. The actual first lactation FCM averages for the various groups are as follows: thirty-seven linebreds, 11,455 pounds, thirty-nine outcrosses, 11,380 pounds and thirty-nine crossbreds, 10,993 pounds. These differences are not significant.

Heifers from each mating system were placed on a standardized ration of free choice alfalfa hay and three pounds of grain per day for 120 days between the ages of 12 to 16 months. Analysis of variance indicated significant differences among groups and among sires within groups for therms of energy consumed in hay and average rate of gain over the experimental period. The

outcrosses gained the most rapidly followed by linebreds, Swiss-Holstein crosses and Ayrshire-Holstein crosses. There were no differences among groups or among sires within groups in efficiency of feed utilization.

A second objective of this study was to determine relationships between growth and efficiency as heifers and production and efficiency as cows. A significant negative correlation was found between therms of energy consumed during the 120 days growing period and fat corrected milk yield for the Swiss-Holstein crosses. Although not significant, the correlations between productive milk traits and growth traits were negative. These data suggest little relationship between growth characteristics and milk production traits. (AH g2-24)

4. Crossbreeding using artificial insemination. At Minnesota a study was conducted to determine the value of crossing breeds using sires as they are available in A.I. In two State Institution owned herds of Holsteins a portion of their cows were mated to Brown Swiss bulls and comparisons were made between the resulting crossbreds and contemporary purebreds. In the Moose Lake herd 17 purebred first lactation heifers produced 10,436 pounds of FCM as compared to 10,345 pounds of FCM for the crossbreds. At Willmar, the purebreds produced 8,694 pounds of FCM as compared to 7,722 pounds FCM for the crossbreds. It is interesting to note the wide differences in results from the two herds. At Moose Lake the crosses were nearly equal to the purebreds while at Willmar the crosses were markedly inferior. The two herds are served by different artificial breeding organizations with different bulls. It appears that the organization serving the Moose Lake herd had Holstein and Brown Swiss bulls of about equal merit while the organization serving Willmar had better quality Holstein than Brown Swiss bulls. Results indicate that no advantage would be gained for dairymen with Holstein cattle to breed their cows to bulls of other breeds. (AH g2-26)

5. Usefulness of heterosis resulting from interbreed matings. An analysis of birth weights of calves born in generations 1, 2 and 3 (1/2, 3/4 and 5/8 of Holstein and Guernsey breeding) from the Illinois project showed that crossbreds were 3.2 lb. heavier than the average of the parental purebreds. For each increase of 1/8 Holstein breeding in the dam, the weight of the calf increased 2.03 lb., while the weight of the calf increased only 0.75 lb. for a comparable increase in the germ plasma of the sire. None of the crossbred groups were as heavy as the purebred Holsteins. There were considerable differences in birth weights associated with sex, parity and sires within breeds, but because of the disproportionate numbers in the breed groups, adjustments had little effect on the sub-class means.

There was an apparent maternal effect on birth weight and paternal effect on gestation period as the 5/8 Guernseys were as heavy at birth as the 5/8 Holsteins. It is likely that the maternal and paternal effects were the results of an interaction between birth weight and gestation periods. When a Guernsey sire was mated to a Holstein female the crossbred fetus had an inherent capacity for a lighter birth weight and a longer gestation period

than the purebred Holstein dam, thus at the time of parturition the fetus had been carried as long as if it were a purebred Guernsey and grown to almost the weight of a purebred Holstein. (AH g2-23 Rev.)

Data on purebred Red Danes, Red Polls, Milking Shorthorns and two- and three-breed crosses in the Indiana project were analyzed for the effect of type of dam (purebred or crossbred), breed of dam, breed of sire, breed of dam by breed of sire interaction, sire within breed, year of calving, and season of calving on milk production, highest monthly milk yield, persistency during lactation and 30-day periods of production.

Highly significant effects were generally found for breed of dam, breed of sire, breed of dam by breed of sire interaction and sire within breed. Significant effects were also generally found for type of dam and season of calving. Other effects were, in general, nonsignificant.

The results of the comparison of purebreds with two-breed crosses indicate heterotic effects for total milk and for yield during the first eight months of lactation, but this came mainly from crosses involving the Red Dane breed. Heterosis was found in all of the crossbreds except for persistency and the last 30-day period of production (10th month) in the case of the Red Dane x Milking Shorthorn crosses. In this study little additional superiority was obtained by crossing two-breed crosses to a third breed. The crosses having the Red Dane breed as one of the parents showed the largest degree of heterosis and they were also the highest groups for milk yield.

Specific combining ability - an estimate of how well specific breeds combine - was unimportant, but the effects from general combining ability - a measure of the overall efficiency with which various breeds combine - were highly significant. The Red Danes were the highest producing breed and had the highest general combining ability with the other breeds. (AH g2-23 Rev.)

A preliminary analysis of rate of gain at three month intervals from birth to 12 months of age for crosses among Ayrshires, Brown Swiss, and Holsteins at Beltsville indicate that the gain of two-breed crosses exceeded the parental mean by 6% during the first 3-month period. This was reduced to 2% during the next six months and the crosses did not exceed the parental mean during the 9 to 12 month period. The larger difference during the first three months was attributed to the slow start of the purebred Swiss. Gains of the crosses exceeded the expected to a significant degree (7%) from 12 months of age through the first lactation. The accelerated gains of the crosses and purebred Swiss at the later ages were due to fleshing rather than an increase in skeletal dimensions.

Studies of various phenotypic traits of body conformation showed that two-breed crosses tended to score higher in most traits than the parental mean. The crossbreds exceeded the parental mean to a significant degree in condition or body fleshing. Some characteristics, such as straightness of rear

legs, shape and attachment of udder, and teat placement were influenced more by crossing than others and breed of sire appeared more important than breed of dam in determining these phenotypic traits.

Thus far there has been no evidence of significant differences between purebreds and crossbreds in the number of services for conception, interval from calving to first estrus, interval from calving to first service, interval from first service to conception and mean calving interval, although the crossbreds were slightly superior in these traits. The crosses required 0.4 less services per conception, came in heat 9 days earlier after calving, conceived 5 days earlier, and had a 10 day shorter calving interval than purebreds. (AH g2-23 Rev.)

A preliminary analysis of production records of purebred Ayrshires, Brown Swiss, and Holsteins and two-breed crosses among the three breeds from the Beltsville project showed no significant difference between purebred Holsteins and Ayrshire x Holsteins and Brown Swiss x Holstein crosses in first lactation total milk, 4% FCM, solids-not-fat, and protein yields, or total therms produced. These two crossbred groups were significantly superior to the purebred Holsteins in milk fat production, body weight gain in lactation, and the number of days carried calf during lactation. The purebred Ayrshires, Brown Swiss, and the Ayrshire x Brown Swiss crosses were similar in milk, 4% FCM, and protein yields, and total therms produced, but were significantly below the other three groups for these traits. The purebred Brown Swiss and Ayrshire x Brown Swiss crosses were significantly higher than all other groups in body weight gains during first lactation. The purebred Ayrshires were lowest in persistency of milk yield, while the purebred Brown Swiss and Holstein x Brown Swiss crosses were highest. There was no significant difference among the six breed groups in gross efficiency (lb. FCM/therm estimated energy consumed) or in the ratio of total therms produced to total therms consumed although the Holstein crosses were slightly superior in these traits. These preliminary results indicate that half-Holstein crosses produce slightly more total energy during the first lactation and do so a little more efficiently than purebreds or Brown Swiss x Ayrshire crosses. (AH g2-23 Rev.)

An analysis of gestation length involving two and three breed crosses with Holsteins, Ayrshires, and Brown Swiss at Beltsville showed a significant maternal as well as paternal effect upon gestation length. The means for Holstein, Ayrshire, crossbred, and Brown Swiss dams were 280, 280, 282, and 285 days, respectively, while the means for Holstein, Ayrshire and Brown Swiss sires were 280, 281 and 285 days. The interaction between breed of sire and dams was also significant. Purebred Holsteins had an average gestation length of 277 days; purebred Ayrshires 279; and purebred Brown Swiss 288 days. Matings between Ayrshire sires and Holstein dams, or their reciprocals, resulted in a mean gestation length of 278 days. Ayrshire and Holstein sires mated with Brown Swiss dams resulted in a mean gestation length of 284 days. Brown Swiss sires mated with either Holstein or Ayrshire dams resulted in a mean gestation length of 285 days.

These results indicate that matings involving Brown Swiss blood caused an increased gestation length.

Similar results have been obtained in the Illinois study where Holsteins and Guernseys were crossed. The means for purebred Holsteins and Guernseys were 279 and 284 days, respectively. The Guernsey dams bred to both Guernsey and Holstein sires carried their calves an average of 283 days, whereas Holstein dams bred to both Guernsey and Holstein sires carried their crossbred calves for 281 days. Calves by Guernsey sires were carried 284 days as compared to 279 days for calves by Holstein sires.

The average gestation length for all crossbred calves at Beltsville and Illinois was the same as the parental mean, indicating that the portion of the breed involved in the mating largely determined the gestation length and that additive gene action, rather than heterosis, is involved. (AH g2-23 Rev.)

Nine body measurements taken at 18, 24, 30, 36 and 48 months of age in the Illinois herd were used to determine the relationship of growth of purebreds and crossbreds to concurrent FCM production and pregnancy. The only significant effects of pregnancy on growth before 24 months were for heart girth and two length dimensions. Both lactation and pregnancy produced their largest effects on growth in the 24-30 month period. In this period all the significant regressions were negative. Fleshing measures (forechest circumference, depth and width) were most severely affected. After 30 months both factors produced fewer significant effects on growth, but lactation had the greater effect throughout. The growth of cows with crossbred dams was least affected by concurrent lactation and pregnancy. Both breed of sire and breed of dam had significant effects on skeletal growth but none of their interaction effects were significant. (AH g2-23 Rev.)

6. Genetic methods for developing adaptability. Phenotypic and genetic parameters were determined from 2,297 records of 615 daughters of 56 sires in the Louisiana State University herd. Intrasure regression estimates for the heritability of milk production per day of productive life, length of productive life, breeding efficiency and type were 0.28 ± 0.12 , 0.00 ± 0.09 , 0.07 ± 0.13 and 0.34 ± 0.16 , respectively. The paternal half-sib estimates were 0.49 ± 0.16 , 0.39 ± 0.14 , 0.11 ± 0.10 and 0.15 ± 0.14 . Significant ($P < .01$) phenotypic correlations were found between ME milk production per day of productive life and 2X-305 day ME first record (0.45), first record and breeding efficiency (-0.24), age at disposal and type (0.39), actual lifetime production and type (0.36), actual lifetime production and first record (0.29), age at disposal and first record (0.22) and length of productive life and first record (0.22). The estimates of heritability and the phenotypic correlations obtained are of comparable magnitude to those for the same traits in cooler climates indicating the expected changes from selection in warm climates is similar to that of cooler climates. (AH g4-2)

A study of 5 years of data on percent solids-not-fat (SNF) of purebred Holsteins, 1/2 Holstein crosses out of Jersey or Red Sindhi-Jersey crossbreds and 1/2 Brown Swiss crosses out of Jersey, Red Sindhi-Jersey crossbreds or Holstein dams in the herd at Jeanerette, Louisiana, showed the average for purebred Holsteins (8.29%) fell below the minimum requirement for most southern milk markets (8.50%); whereas the crossbred groups were well above, 8.72 and 9.00% for the Holstein and Swiss crosses, respectively. The orthogonal comparison of Holsteins vs both crosses and Holstein crosses vs Brown Swiss crosses were both highly significant ($P < .01$). The interaction of year-season with breed groups was nonsignificant. The difference in percent SNF between the two crossbred groups was primarily due to breed of sire since the mean SNF for dams of the crossbreds was about the same. The average percent fat was 3.52, 4.03 and 4.02 for the purebred Holsteins, Holstein crosses and Swiss crosses, respectively. The purebred Holsteins averaged 645 lb. milk more than the 1/2 Holstein crosses and 861 lb. more than the Swiss crosses. (AH g4-2)

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DAIRY CATTLE - PHYSIOLOGY
Animal Husbandry Research Division, ARS

Problem. Fundamental physiological research is required as a basis for improving lactational and reproductive performance of cattle. Breeding failure is a major reason for the disposal cattle. Further information is required on the physiological action of hormones in controlling reproductive activity, correcting reproductive abnormalities and stimulating lactation. Research on physiological processes related to growth and development, nutritional requirements and to heat tolerance of dairy cattle is also required.

USDA AND COOPERATIVE PROGRAM

This is a continuing program, almost entirely on basic research, conducted by physiologists and biochemists. The program is designed to elucidate the reproductive and lactational physiology of cattle utilizing physiological biochemical techniques and to determine physiological mechanisms related to heat tolerance. The work is in progress at Beltsville, Maryland, and co-operatively at the Wisconsin, New York, Massachusetts, Texas, Louisiana, Georgia Agricultural Experiment Stations. It is coordinated with the NE-41, W-49, and S-49 regional projects.

The Federal scientific effort devoted to the research in this area totals 7.7 professional man-years. Of this number, 2.9 are devoted to the physiology of reproduction, 2.0 to the physiology of milk secretion, 1.0 to the physiology of growth and development, 1.3 to environmental physiology, and 0.3 to program leadership.

A grant with the Veterinary School of the University of Sao Paulo, Brazil, provides for research on the anatomical and physiological characteristics affecting heat production and heat loss of Zebu, European and Zebu-European crossbred cattle and the nature and method of controlling the inheritance. Its duration is for five years, 1961-66, and involves PL-480 funds with a \$63,293 equivalent in Brazilian Cruzeiros. (Pertains to Area 5 also).

A grant has been initiated with the Veterinary Institute, Beit Dagan, Israel, in the Department of Reproduction, for work on a project entitled, "Comparative Studies of Repeat Breeders and Normal Cows and Heifers." It is for a five-year period and involves PL-480 funds to the extent of \$124,600 equivalent in Israeli pounds.

A grant was initiated with PL-480 funds with the Department of Applied Pharmacology, The Hebrew University, Rehovoth, Israel, on the mechanism of lactation and its augmentation by hypothalamic stimulation. It is supported for five years and is for \$126,767 equivalent in Israeli pounds.

PROGRAM OF STATE EXPERIMENT STATIONS

Current physiological research can be divided roughly into four categories, viz., digestion (especially rumen function), reproduction, milk secretion, and all other research. Several states are determining the major factors which influence the ratio of volatile fatty acids (VFA) produced in the rumen: some are determining the metabolic changes which occur before the VFA reach the blood stream; but only a few scientists are concerning themselves with the manner in which these VFA are utilized by the cow (intermediary metabolism). This is not because intermediary metabolism is not an important field of research but because of the scarcity of trained scientists. Digestion trials per se are receiving less attention than formerly.

A large amount of endocrine research is underway, especially as it relates to reproduction. Fewer workers are injecting the sex hormones promiscuously, or assaying the endocrine glands for their hormone content, and more are attempting to measure the hormone titer of the venous blood leading off from a ductless gland. Scientists at a few schools have become interested in the mechanisms which modify the normal secretion rate of endocrine glands, such as the role of the nervous system, the hypothalamus and the uterus. Such studies also include factors which modify the sensitivity of a target organ or tissue to the hormone(s) involved. Ova transfer, lyophilization of sperm and ova, what constitutes capacitance in spermatozoa, and how it relates to viability, plus many other studies, constitute the field of reproductive physiology.

Much endocrine research outside the field of reproduction also exists. The secretion rate of endocrine glands in vivo, especially the thyroid, the role of the parathyroid in milk secretion, especially the ability of the parathyroid to prevent milk fever, and factors which influence appetite are typical of these extra-reproductive endocrine studies. But the most important research in the field of endocrinology outside of reproduction is concerned with milk secretion. Innumerable other physiological reactions of the cow are being closely observed: temperature control, metabolism (detoxification) of insecticides and other poisons, resistance to disease, etc.

The total research effort on physiology of dairy cattle in 42 states is approximately 52.4 professional man years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Physiology of Reproduction

1. The corpus luteum. Human chorionic gonadotropin (HCG) was used to induce ovulation in ewes. Ewes that had their natural corpora lutea (CL) removed at the time of treatment with the ovulating dose of HCG had cycle lengths of 20 days. The new glands thus were formed in the relative absence

of progesterone. Ewes treated with HCG that had their natural CL left in the ovary for 2 days and then were removed had estrual cycles of 14 days, as did a group treated with HCG, natural CL removed but treated with progesterone. The data suggested that the presence of progesterone at the time new corpora are induced during the estrual cycle acts to shorten the life-span of such CL in ewes.

Cows were studied before and after the time of parturition to determine more exactly certain pituitary-ovarian relationships. The prepartum CL had a progesterone content of 26 $\mu\text{g/g}$ and were larger than CL on the day of calving which had no detectable progesterone. The pituitaries of the day-of-calving group had a significantly higher FSH level and lower LH level than the prepartum group. Injection of progesterone did not produce significant alterations in the FSH and LH levels of the pituitary glands.

In vivo treatment with oxytocin, glucose or both had no significant effect upon CL weight, initial progesterone concentration or synthesis of progesterone during incubation of luteal slices. When pregnenolone was added to the luteal slices, glucose, either in vivo or in vitro, increased the conversion of pregnenolone to progesterone ($P < 0.05$). The combination of both in vivo and in vitro glucose treatment caused a further increase in production of progesterone from pregnenolone. (AH h5-6)

Slices of bovine CL obtained during the estrous cycle were incubated for 2 hours in the presence and absence of luteinizing hormone (LH). LH stimulated progesterone synthesis and glycolysis in all CL with the exception of those obtained near the end of the estrous cycle. Progesterone synthesis in CL obtained day 19 or later can be restored by adding pregnenolone to the medium, suggesting that failure of pregnenolone formation may be responsible for the cessation of progesterone synthesis associated with luteolysis. (AH h5-4)

The mechanism by which hysterectomy prolongs the functional life of the corpus luteum was studied in heifers and rats. Both the CL and uteri were removed from heifers at the 9th-11th day of the estrous cycle. Such animals were capable of forming new CL, indicating that removal of the uterus does not block follicular development and ovulation.

Since cavernous sinus blood drains the pituitary, blood collected from the cavernous sinus may contain levels of pituitary hormones which can be related to pituitary and gonadal function. A technique was developed which consists of passing a needle through the foramen ovale into the cavernous sinus while the cow is restrained in an operating stall with its head tied to one side. Up to one liter of blood may be collected from each cow, heifer or calf. (AH h5-3)

2. The oviduct in sperm and ovum transport. Estrogen administered to rats on days 1-3 post-coitum or on day 3 post-coitum was found to interrupt pregnancy. The data suggest that pregnancy was inhibited by hastening the transport of ova into the uterus. In related studies on oviduct fluid secretion in sheep, the mean secretory rate of oviduct fluid at estrus was 2.3 times greater than 10 days after estrus, when it was at its lowest point. (AH h5-4)

3. Contraceptive action of intrauterine plastic devices in cattle and rats. Polyethylene spirals or loops were placed in the lumen of both uterine horns of parous Holstein cows. Small loops were expelled from the uterus but when larger loops were used they were completely effective in preventing pregnancy. Corpus luteum development was inhibited in about 1/3 of the estrous cycles. Cystic ovarian follicles developed in some of the cows. Slaughter studies suggested that the plastic devices in cattle uteri exert their contraceptive effect in large part by interfering with reproductive processes before ova reach the uterus.

In related experiments with rats, a thread was placed in one uterine horn to mechanically induce infertility and the rats were mated. No pregnancies occurred in the horns containing the contraceptive thread although the ovary on this side had ovulated to as great an extent as the contralateral ovary, as assessed by corpora lutea counts. Normal embryos were present in the non-thread horn in about the same frequency as normal rats. (AH h5-8)

B. Physiology of Milk Secretion

1. Histamine excretion of cattle fed various rations. Previous studies on the histamine content of cattle blood, milk, and urine demonstrated a much higher urinary histamine in heifers fed silage than in animals fed hay. Since silage feeding is often associated with an appetite problem, investigations were initiated to further characterize the role of naturally occurring histamine in various cattle rations upon the histamine concentration of rumen contents, feces, and urine. Examination of the feedstuffs, alfalfa hay, orchardgrass silage, and corn silage, revealed that by far the highest concentration of histamine was found in corn silage. The concentrations of histamine found in the urine and feces of cows receiving corn silage were several times greater than those found in the excreta of cows fed grass silage, and the grass silage levels were about twice that of the animals fed hay. In another study fistulated heifers were used and the histamine concentration determined in rumen contents, feces and urine when the animals were on various rations. A high urinary level, 127 µg/ml was found during corn silage feeding which decreased to 2 µg/ml on alfalfa hay. The dry matter feed intake was as much when fed corn or grass silage ad libitum as when fed hay, thus suggesting that the high amounts of histamine in the ration were not associated with a factor reducing intake. (AH h5-1)

2. Glycogen in normal and mastitic milk. Earlier studies demonstrated that mammary glands with induced mastitis had a higher tissue glycogen concentration than non-infected glands. Studies on milk from 11 mastitic cows demonstrate that the milk from mastitic quarters of the udder have a higher concentration of anthrone-positive material (glycogen) than milk from the non-infected quarters of the same udder. The glycogen concentration of mastitic milk was 7 times that of the normal milk ($P = 0.01$). A method for the disaccharide, lactose, which would effectively separate it from any monosaccharides present and from larger polymers, such as glycogen, was developed. (AH g3-8)

3. The mechanism of lactation and its augmentation by hypothalamic stimulation. The interrelationship of the hypothalamus and the interior pituitary gland in stimulating lactation was studied by depressing the activity of the hypothalamus with tranquilizers and other drugs. The research conducted this year demonstrated that milk producing neurotropic drugs are not necessarily hypothalamic depressors since antidepressants did not abolish the milk secretion. Culture of pituitary and hypothalamus together revealed that prolactin release was increased by perphenazine, estradiol and hydrocortisone. Estradiol and hydrocortisone act directly on the hypophysis whereas the action of perphenazine is mediated by the hypothalamus. Prolactin release was not affected by testosterone or oxytocin but was suppressed by estriol. Perphenazine, which was superior to chlorpromazine in eliciting hypothalamic lactation, was excreted in the milk. Tritium labeled compounds showed radioactivity in the milk equivalent to concentrations in the plasma. (A10-AH-3 Israel)

4. Effect of autoimmunization with semen on sperm production. At Beltsville, seven young Holstein bulls were put on a semen collection schedule of three times per week. After control periods of 2 months or longer, semen injections were started. Six bulls were injected intradermally each week with their own semen mixed with complete Freund's Adjuvant. The seventh bull was injected with the adjuvant and saline. After four months of treatment, the bulls showed no evidence of an effect on sperm or semen production. Small but significant amounts of anti-seminal plasma antibody were found in the blood of each of the bulls injected with semen. The control bull did not produce anti-seminal plasma antibodies. In three of the bulls which were slaughtered no evidence of tissue-fixed antibody was found. Rabbits injected with semen from each of the bulls all produced high titers of anti-seminal plasma antibodies. (AH g1-7)

C. Environmental Physiology

1. Effect of high fat rations and heat stress on milk production, milk composition and rumen acids. Three rations consisting of (a) alfalfa hay and concentrate; (b) hay and concentrates containing 10% soybean oil; and (c) hay and concentrates containing 10% hydrogenated vegetable fat were fed to lactating cows during alternate two week periods of cool (60-75°F.) and hot (90°F.) environmental temperatures. Under the cool conditions ration (b) caused some decline in milk fat percent and a decrease in the rumen acetate-propionate ratio. The consumption of ration (c) was lower, but it increased milk fat percent slightly. The hot

environment decreased feed intake (20%), milk production (30%), milk fat percent (29%) and caused a significant decline in percent solids-not-fat and protein. There was some tendency for recovery in milk composition during the second week of heat stress. No significant differences were observed among rations under hot conditions in respect to milk yield or composition, although the soybean oil ration tended to produce more FCM. Total rumen acids were not significantly altered although there was an increase in the acetate-propionate ratio under the hot environment.

High fat feeding either as hydrogenated vegetable fat or soybean oil had little effect as compared to temperature on various physiological changes, although the cows receiving the hard fat ration had slightly higher body temperatures and respiration rates at the 90° temperature and rations x temperature interactions were significant for urine volume and specific gravity and hair coat depth.

At 90°F. there were significant decreases in body weight, urine specific gravity and pH; hematocrit % and respiratory tidal volume. There were significant increases in water consumption; urine volume; body temperature; respiration rate; respiratory volume, respiratory evaporation and insensible weight loss. The greatest changes occurred during the first week in all cases with some indication for recovery in most traits by the end of the second week. Hair coat depth and the number of red and white cells were not significantly influenced by the environmental changes. Among cow variance was important in the changes in most traits under the hot conditions indicating that the individual cows responded somewhat differently to the rations and changes in environment.

Under the conditions of this study, there was no clear evidence that the feeding of high levels of fat made the lactating cows more comfortable under heat stress. (AH g4-1)

2. Heifers can adapt to prolonged heat stress. Animals subjected to prolonged heat stress commencing in the winter months eventually adjusted their body temperatures to normal levels, whereas, this did not occur in the "summer conditioned" animals exposed to the same level of environmental temperatures. To study the physiological adjustments "winter-conditioned" animals made to heat stress, six three-year old Hereford heifers were subjected to a constant temperature of 90°F. and 60% relative humidity for 95 days commencing in January. The heat stress caused a significant change in all the physiological responses measured, but by the 95th day all had either plateaued at a level different from the preliminary period or returned to near normal levels. In most cases a readjustment was evident by the 40th day.

The average daily gain during the preliminary period was 1.79 lb. but declined to 0.62 lb. by the end of the 3rd week and to 0.23 lb. by the end of the 6th week. During the last 6 weeks, gains increased to 0.67 lb. and reached 0.75 lb. during the three-week post experimental period. The lower rate of gain

during the post period was partially due to the lowered level of feeding as the animals were about 70 lb. heavier. The reason for the marked decline in the rate of gain during the stress period is not clear as feed consumption never declined more than 10%, and for 10 of the 13 weeks the animals consumed all they were offered. There were neither significant changes in the digestibility of the dry matter nor in the total bacteria counts in the rumen during the heat stress period. Apparently, energy was used, at least partially, in combating the effects of the heat stress (ex., the energy expended in high respiration rates). (AH g⁴-1)

3. The relationship of physiological responses to blood and rumen constituents under heat stress. Multiple regression methods were used to determine the relationship of rectal temperature; respiration rate; water intake; hematocrit percent; and hair coat depth to blood and rumen constituents when animals were subjected to a 90°F. environmental temperature. The percent of variation (R^2) accounted for by these variables ranged from zero to 38% but there was little evidence of consistent significant variation being accounted for by the individual variables.

From this analysis it does not appear that rectal temperature, respiration rate, hematocrit percent, hair coat depth, and water consumption can be used effectively in estimating the changes that will occur in blood constituents and rumen acids under heat stress. (AH g⁴-1)

4. Adaptive responses of Holstein heifers to controlled and natural climatic conditions. At Louisiana State University, adaptive responses were studied in two groups (five animals each) of 12- to 15-month-old virgin Holstein heifers under controlled and cycled hot and cool and under natural climatic conditions.

Mean sweating rates from the forechest of the heifers by the capsule technique at air temperatures of 66, 77, 85, and 96°F. were 86.0, 107.2, 138.8, and 210.0 g/m²/hr., respectively. At 101°F. and 29 mm Hg animals previously acclimatized to summer conditions had an average sweating rate of 280.8 g/m²/hr. as compared with 132.0 g/m²/hr. for those previously exposed to cool conditions. The average number of sweat glands per cm² of skin by the biopsy technique was 991 ± 89. There was a significant correlation of 0.645 between sweating rate and the daily gain in weight.

The averages for daily body weight gain during the spring and cycled hot periods were 2.29 and 0.92 lb., respectively. Under the air conditioning and natural summer climatic conditions, the averages were 2.19 and 1.80 lb., respectively. (AH g⁴-1)

5. Studies of heat tolerance of Indian cattle and buffaloes. The study of responses of Haryana, Kankrej and Tharparkar breeds of cattle and Murrah buffalo to heat stress and humidity are being conducted at the Indian Veterinary Research Institute, Izatnagar, India, under a PL-480 grant. These studies are conducted in a psychrometric chamber under environmental conditions

consisting of cool (70°F.); hot-arid (120°F.), with 15% humidity; and hot-humid (105°F.), with 70% humidity.

Preliminary data indicate that exposure to both the hot-arid and hot-humid environments resulted in significant increases in respiration rate, respiratory volume, rectal temperature and pulse rate as compared to the cool conditions. Breed differences were not significant. The buffalo responded very similarly to cattle with few exceptions. The buffalo had slightly lower pulse rates under all conditions studied. The rectal temperature of the buffalo was at its highest under the hot-arid conditions, whereas the cow rectal temperature readings were highest under the hot-humid conditions.

Evidence thus far indicates that other variables will need consideration for fully differentiating the physiological characteristics of Indian cattle and buffaloes. (A7-AH-1)

6. Adaptability studies in Brazil. Commercial dairy herds in the Sao Paulo milk shed containing purebred or high grade Brown Swiss, Jerseys, and Holsteins were studied. The mean ages at first calving were 29, 35, and 41 months for Jerseys, Holsteins and Brown Swiss, respectively. The average time from calving to conception was 3.8, 4.3 and 4.7 months for Jerseys, Holsteins and Brown Swiss. The mean calving interval for the three breeds ranged from 14 to 17 months and gestation periods ranged from 274 to 286 days with the Brown Swiss having the longest interval in each case. There was no evidence of breed differences for length of time in the herds. Jerseys had the highest reproductive efficiency, but Holsteins excelled in milk yield per day of life. (S3-AH-7)

Twenty heifers (10 Zebu and 10 Zebu x European crosses) were kept in the psychrometric chamber for three month periods under each of the following assimilated seasonal conditions: spring (68°F.); summer (80°F.); fall (66°F.); and winter (54°F.). At the end of each of the three month periods the temperature was raised to 104°F. to determine the effect of heat stress on heifers accustomed to various seasonal conditions. There were significant seasonal effects reflected in the number of red and white blood cells, hemoglobin, hematocrit, body temperature and respiration rate. Seasonal effects were also important in the level of response at 104°F., particularly in the blood constituents.

This study indicates that the level of response to heat stress is largely dependent on the conditions the animals are exposed to prior to heat stress and also shows that among animal response to heat stress may be more important than among breed response. (S3-AH-7)

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DAIRY CATTLE - NUTRITION AND MANAGEMENT
Animal Husbandry Research Division, ARS

Problem. Information on the nutritional processes and requirements of dairy cattle is needed to obtain a more precise evaluation of feeds and rations as a basis for improving feeding practices on farms. Shifts in sources of nutrients fed to dairy cattle require studies on the optimum combination and specific supplements needed in order to provide for the most profitable production. Also, dairymen need to reduce costs including man-hours of labor and develop better management in the use of improved types of dairy equipment and feeding, bedding, and milk handling systems.

USDA AND COOPERATIVE PROGRAM

The current program is conducted by biochemists, nutritionists and dairy husbandmen. At Beltsville, studies are in progress on the relationship between net energy, metabolizable energy, and total digestible nutrient values in dry roughages, silages, green roughages, and concentrates; and the relationship between digestibility and the chemical composition and solubility of various feed constituents. Calorimetric techniques are being applied to studies on the effects of dietary and physiological factors on energy metabolism and requirements of cattle. A cooperative project at Tifton, Georgia, has been initiated on the residues in milk resulting from the ingestion of pesticides and herbicides associated with the treatment of animals and crops.

At Beltsville, Maryland, research is being conducted on the effects of crop maturity, moisture content, preservatives, including methods of handling and conditions of storage, on the chemical quality, palatability and feeding value of silages. In conjunction with this effort, biochemical studies are being made to determine the effect of the composition of forage at the time of ensiling and of varying imposed conditions on the composition of the resulting silage. Related to the Beltsville studies is cooperative work at Lewisburg, Tennessee. The objective of the work at the Tennessee station is to determine the effectiveness of various practical ensiling procedures by varying such factors as moisture, preservatives, type of silo, etc. Pasture studies at Beltsville, Maryland, involve the effect of varying stocking rates on nutrient yields per acre and on production per animal.

A cooperative project at Logan, Utah, has been undertaken to measure the variations in efficiency of forage utilization by dairy heifers and to determine the factors which account for these variations.

The work at Beltsville, Maryland, also consists of studies on wilted silage as a forage for growing dairy heifers, the vitamin and mineral requirements of calves and deficiency symptoms using a synthetic type of diet with particular emphasis on vitamin A and magnesium deficiency.

Scientists at Beltsville are engaged in studies on the environmental conditions and the mechanisms of infection involved in bovine mastitis. In cooperation with Agricultural Engineering, Entomology, and Eastern Utilization, research is in progress on electrically-controlled and operated equipment for reduction of labor in dairy cattle management; on the evaluation and development of physical methods for control of flies and other dairy cattle pests; and on the relationship between management practices and milk quality including flavors.

Cooperative work with Agricultural Engineering and with the Georgia Coastal Plain Experiment Station is being conducted on the influence of management practices and other environmental factors on the adaptability of cattle to the Southeastern United States.

The Federal scientific effort devoted to research in this area totals 25.4 professional man-years. Of these 7.0 are in digestion and metabolism, 8.1 in forages, 3.1 in nutritional requirements, 3.6 in calf feeding, 2.7 in management practices, and 0.9 in program leadership.

A grant with the Government Agricultural College and Research Institute, Ludhiana, which is affiliated with Punjab University, Chandigarh, Punjab, India, provides for research on factors affecting the utilization of low-grade roughages and production of volatile fatty acids in the rumen of cattle. Its duration is for five years, 1962-67, and involves PL-480 funds with a grant of \$86,598 equivalent in rupees. (Pertains to Area 1 also).

A contract in the amount of \$10,000 per year to evaluate the feeding value of newer corn hybrids is in effect at the Maryland Agricultural Experiment Station. Duration four years.

A contract in the amount of \$25,000 per year to study the effects of level of concentrate feeding on cost of milk production is in effect at Cornell University. Duration four years.

PROGRAM OF STATE EXPERIMENT STATIONS

Calves. At what age and why do calves stop absorbing antibodies from the colostrum is still a moot question. Efforts are being made to reduce the milk feeding period. Different levels and kinds of vegetable oils along with emulsifiers, are being tried, especially for veal production. The newer antibiotics as well as digesters (enzyme preparations) are being studied. Others are interested in the synthesis of the B-vitamins in the intestine, in dextran as a source of iron, and still others in the zinc and manganese requirements of young calves.

Pregnancy and lactation. Much more emphasis is now being placed on the condition of the cow at time of freshening including the permeability of the placental membrane to nutrients circulating in the maternal blood stream. Almost every station is still testing the effects of high quality forage

on milk production, or in vitro with an artificial rumen. The validity of the Morrison feeding standards are being questioned for high producing cows. As a result many experimental animals are being challenged with liberal grain feeding immediately on freshening. The effects on the composition of the milk of high grain feeding, including processing of the grain, are also under investigation. Efforts have also been made to increase the fat in the ration, especially unsaturated fats, in the hope of not only producing milk more economically but with more polyunsaturated fats present. High fat rations might also be less heating.

Copper compounds have been injected intravenously to see if they influence the susceptibility of cow's milk to become oxidized. Fluorine has been fed at various levels over long periods of time. There is also the problem of nitrogen fertilization on the availability of magnesium in forage, and of nitrates in the plant on the vitamin A reserves after feeding, including their effect on reproduction. What is the volatile compound in ladino clover that sometimes imparts an undesirable flavor to milk? Can these suspected compounds be tagged with a radioactive compound and thereby be traced and identified more readily? Will drugs like tapazole, thyroxine, diiodosalicylic acid, nitrofurazone and many others benefit the lactating cow?

Research on the use of milking machines, automatic feeding devices and free stall housing will be discussed in another section.

The total research effort on dairy nutrition and management at 43 states is approximately 76.4 professional man years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Digestion and Metabolism

1. Energy Metabolism Laboratory. The major emphasis in the research program for the past year has been to conduct complete carbon, nitrogen and energy balance studies with dairy cows when they were pregnant, non-pregnant, lactating, dry, fattening, fasting, and at maintenance.

(a) Improvement of energy balance methods and procedures.

Additional progress was made during the year to reduce the time and labor required to calculate and summarize the results of respiration trials and energy balance experiments. A computer program for calculating the gas composition from the meter readings of the infrared and paramagnetic analyzers was prepared, and has resulted in the saving of manpower plus increased accuracy.

The method of listing the results of the computations of carbon, nitrogen and energy balance trials and digestion trials was modified so that they are suitable for publication without any transfer or typing of tabular material. An example of this method of preparation of data for publication was prepared and the proposal that this procedure be used in reporting

energy metabolism studies from other laboratories to facilitate completeness of publication, reduce costs of publication, and alleviate language difficulties is being considered by a committee appointed at the 3rd Symposium on Energy Metabolism, held May 19-22, 1964, in Troon, Scotland.

Specifications and plans for a digital data logging system to record data collected during respiration trials were prepared. The system will replace the present automatic data collecting system which is being rented. It will collect, record on typewriter, and punch into cards the data on gas flow, temperature, relative humidity, barometric pressure, animal activity, body temperature, respiration gas composition, and all necessary identification information from 6 respiration chambers. An alarm system to be used in conjunction with the digital data logging system has been installed, which has alleviated the necessity of having personnel in the laboratory at night and on weekends.

(b) Effect of ration composition on efficiency of utilization of metabolizable energy for milk production. Respiration trials and complete energy balance measurements with 7 lactating and non-lactating Holstein cows were conducted. The major purpose of the series of experiments was to determine the influence of ration composition on the efficiency of utilization of metabolizable energy for lactation. The rations were 100% alfalfa (ration C), 75% alfalfa (B), and 50% alfalfa (A), with the remainder of the estimated net energy being supplied as a concentrate mixture of corn and soybean oil meal. Results of the trials showed an increase in the efficiency of utilization of metabolizable energy for lactation and each additional substitution of concentrates for alfalfa on an equal estimated net energy (ENE) basis.

The efficiency of utilization of ME available for milk production, calculated by regression analysis of the data on a between animals basis, was $45 \pm 5.6\%$ for all alfalfa hay, $52 \pm 5.1\%$ for 75% alfalfa, and $56 \pm 4.2\%$ for 50% alfalfa. Using metabolizable energy consumed (ME_0) as the dependent variable, the efficiencies were 51, 56 and 58% for rations C, B, and A, respectively.

The above values were all considerably below what would have been expected based on previously reported experiments with lactating cows. However, the efficiency values can be markedly influenced by the assumptions made concerning the maintenance requirements and factors used to correct to zero energy balance. The data were computed assuming the maintenance requirement to be $131 \text{ kcal ME/W}^{0.75}$ and the correction factors for tissue deposition loss or gain to be $1.61+$ or $1.43-$. Using this approach, greater apparent efficiency values (54-65%) resulted, but the same conclusions regarding the influence of ration composition on the utilization of energy for milk production were reached. The mean efficiencies for converting available ME to milk were 65%, 61%, and 54% ($P < 0.05$) for rations A, B, and C, respectively.

The data were also computed by using covariance analysis to adjust to a common milk energy output. These computations showed that more ME was required for milk plus maintenance from the all-alfalfa hay ration than was required from a ration of alfalfa and concentrates when the ME was regressed on milk energy. This method of computation does not require the use of an assumed maintenance requirement, but it does not distinguish between efficiency for lactation and efficiency for maintenance.

(c) Utilization of metabolizable energy. The maintenance requirements of the lactating cows, calculated by regression, were 110, 110 and 109 kcal ME/W^{0.75}_{kg}/24 hr., respectively, for the three rations. All the above values were obtained using constants derived from data obtained within the experiment when the same animals were dry and non-pregnant, to correct for tissue gain or loss. The overall efficiency of utilization of the metabolizable energy of these rations for lipogenesis was $50.2 \pm 3.6\%$. The efficiency of utilization of metabolizable energy consumed above maintenance by dry cows varied from 45 to 56%, depending on the proportion of concentrates in the ration, with the higher efficiencies being on the rations containing concentrates.

(d) Relationships between rumen volatile fatty acids and efficiency of utilization of metabolizable energy. Samples of rumen contents were collected following each of the balance trials and were analyzed. The acetic acid present in the rumen declined from 71.4 to 65.3% ($P < .05$) as alfalfa hay energy was replaced with concentrate energy. A non-significant increase in propionic acid from 16.0 to 18.4%, and an increase in butyric acid from 7.9 to 10.4 molar percent occurred. The correlations between volatile fatty acid proportions and lactation efficiency calculated using an assumed maintenance requirement of 131 kcal/W^{0.75}_{kg}/24 hr. were: $r = -0.73$ for acetic acid, $+0.43$ for propionic acid, and $+0.67$ for butyric acid ($P < 0.05$).

These results would indicate that the reason for the increased efficiency of utilization of feed energy when concentrates replaced forage in the ration was because there was a greater proportionate production of propionic and butyric acid than of acetic acid in the rumen. Thus the type of feed fed can affect the efficiency of feed utilization through the type of fermentation produced in the rumen.

(e) Theoretical fermentation balance computations using respiration trial data. A theoretical fermentation balance equation derived by Wolin (J. Dairy Sci. 43:1452. 1960) was used to calculate the moles of fermented carbohydrate from data collected during energy balance experiments with lactating dairy cows. The proportions of the volatile fatty acids in the rumen of cows being fed rations varying in hay to grain ratios were incorporated into the balance equation. The total methane produced was measured during the respiration trials and was used to quantitatively estimate the moles of hexose sugar fermented for the three rations.

Methane production ranged from 266 to 536 liters per 24 hr., decreasing from 442 liters when the cows consumed 100% alfalfa hay to 409 liters when 50% of the ration ENE was concentrates. The quantities of hexose theoretically fermented were 5.246 kg for the all-alfalfa hay ration (ration C), 5.418 kg for ration B (75% hay), and 5.292 kg for ration A (50% ENE as alfalfa). These corresponded to 81.0%, 84.3%, and 86.4% of the actually determined digestible carbohydrates which were theoretically fermented.

The apparent digestible carbohydrates of alfalfa hay contain pigments, organic acids and tannins which are not fermentable. When corrections were calculated for the unfermentable portion of the alfalfa in each of the rations, carbohydrates which were actually fermented were 100% for ration C, 99.8% for ration B, and 101.9% for ration A. These computations suggest that all of the readily fermentable carbohydrate of each ration was actually fermented. However, many assumptions were made in deriving these results and further data will be needed to substantiate their validity.

(f) Practical significance to date of the energy laboratory studies. Total digestible nutrients (TDN) has been the usual method of expressing the energy values of feeds in the past. When comparing concentrates with each other relatively, the TDN values are essentially satisfactory. However, when comparing the replacement value of concentrates and forages with each other there will be considerable error. This error assumes considerable importance in purchasing feeds and in comparing the relative energy production of forages and concentrates per acre on the farm. The results of these experiments will in the future influence nutritionists to express the energy values of feeds in some terms based on the net energy concept. (AH h2-8)

2. Development of chemical methods for determining the nutritive value of feeds and forage. The nutritive value of forages varies dependent upon stage of maturity, species, method of preservation and fertilizer practices. It is highly desirable that suitable chemical methods be developed that will predict the nutritive value of forages. The previous report discussed new methods for the determination of acid-detergent fiber and lignin.

(a) Cell-wall constituents. A new method has been developed which divides the dry matter of forages into two fractions: cell-wall constituents, which represent the total of the fibrous fractions--lignin, cellulose, hemicellulose and fiber bound protein--, and the cell contents which contain the easily soluble matter that is rapidly digestible. The cell contents can be shown to be virtually completely digestible in ruminants and comprises 20 to 65 percent of the dry matter of forages. This new method differs in both purpose and function from the previously developed acid-detergent fiber (lignocellulose).

(b) Studies on the manner in which lignin affects digestibility. Statistical analyses, involving the form of mathematical expression of lignin in relation to the digestibility of fibrous fractions, showed that

lignin controls the availability of cell-wall constituents only, and has no effect on cell contents. On the other hand, lignin does control the digestibility in direct relation to which the concentration occurs in lignocellulose (termed lignification). The relationship is curvilinear and suggests a first order relationship between lignin and digestibility.

(c) Index of availability. Theoretical considerations have shown that the nutrients in forage are present in two different states that contribute to the resultant digestibility, the amount of nutrients in a state of lignification, and the amount of free cell contents which are independent of the effects of lignin. These two states can be expressed in a ratio as a percent and subtracted from 100 to give an availability index A.

$$\text{Availability Index} = 100 - \frac{\text{Lignin}}{\text{acid-detergent fiber (100-cell wall constituents)}} \times 100$$

Regression equations have been calculated from a group of 40 feeds including grasses, legumes, concentrates, mixtures and straws with the new availability index (A) with correlations of +0.94 with digestible dry matter, +0.05 with TDN, +0.95 with estimated net energy, and +0.96 with digestible energy.

Thus the nutritive value of all classes of feeds can be predicted with some accuracy by means of chemical analysis which has hitherto been impossible. The nutritive value can be expressed as digestible energy or estimated net energy. These new methods will undoubtedly replace methods now being used in the forage testing laboratories in the various states.

(d) Studies on voluntary intake. A total of 121 forages on which voluntary intake has been measured have been analyzed chemically. The relationships of lignin and acid-detergent fiber with voluntary intake reveal intense species differences. The only consistent relationship that can be drawn across all forages is that of the total fibrous fraction, cell-wall constituents (CWC). As this fraction increases, voluntary intake declines with an increasingly negative slope. In forages with a low CWC, digestibility and intake are not apparently related. In forages with a high CWC, intake is highly correlated with both chemical composition and digestible dry matter. This suggests that the relationship between digestible dry matter and voluntary intake depends on the proportion of total digestible nutrient from cell-wall constituents. Accuracy of prediction from the regression of cell-wall constituents on voluntary intake is poor for high quality forage and possesses some precision in low quality forages.

(e) Weighing techniques and sample preparation. A procedure for the weighing of hot crucibles and samples directly from the drying oven has been perfected and eliminates the use of dessicators causing a considerable saving of time. A technique using acetone-drying to prepare wet samples in a dry state without the use of heat has been developed for the purpose of avoiding

formation of lignin artifacts. This adds considerable to the accuracy of the lignin method as well as the accuracy of interpretation which can be placed on the values obtained. (AH h2-6)

3. Chemicals in milk. Dimethoate toxicity. In 1961 at Tifton, Georgia, a corn crop was sprayed with dimethoate, made into silage and fed to lactating dairy cows. During the feeding trial some of the cows on the higher intake levels of 0.13 and 0.28 mg/Kg/day displayed toxicity symptoms including blood clots in the feces and chocolate colored urine. In order to gain further information in respect to the toxicity of the insecticide, alfalfa was sprayed at Beltsville with 2 lb/acre (about 4 times the recommended level) and harvested as silage with the application of 100 lb sugar per ton of fresh material. The resulting silage containing 54-73 ppm of residue was fed to three cows. One Jersey died after 41 days while consuming 2.04 mg/Kg/day, and a second Jersey had to be sacrificed after 44 days while consuming 3.45 mg/Kg/day. A third cow, Holstein, consumed 2.55 mg/Kg/day for 93 days and failed to display any toxic symptoms.

The amounts of dimethoate consumed at Tifton and at Beltsville is below the toxic level as previously determined elsewhere. However, it is known that the oxygen analog of dimethoate is about 10 times more toxic than the dimethoate itself. Whether the oxygen analog or other decomposition metabolites are present and peculiar to silage is not known. Further work is required and is being conducted at Tifton in respect to the use of dimethoate on crops to be made into silage. (AH h2-9)

B. Forage Evaluation and Utilization

1. Effect of moisture level on preservation and feeding value of alfalfa silage. Previous work has shown that alfalfa stored at about 55% moisture content in conventional tower silos was equal in feeding value to barn-dried hay. Further work was required to determine the optimum moisture level for storage and feeding value. Alfalfa forage was harvested and stored in conventional tower silos at three moisture levels 60%, 50%, and 40%. Storage losses were least for the 60% silage and greatest for the 40% silage. Spoilage was a general problem in the 40% silage. Feeding value of the 50% moisture silage was highest on the basis of intake, milk production, liveweight gains, and digestibility. Simple correlations indicated that at moisture contents higher than 50%, the chemical changes in the silage were less desirable (increased butyric acid and lowered sugar content) and intake was lower.

It was concluded that an average moisture content between 50 and 60% is optimum for storage in conventional silos and that the intake and feeding value of the silage will be equivalent to barn-dried hay. Thus the difficulty previously encountered of lowered intake of dry matter with high moisture silage (60% moisture and above) has been overcome by procedures which can be fully automated and without the need for special equipment. (AH h3-3)

2. Low-moisture grass silage stored in a bunker silo. Previous studies of low-moisture silage have been confined for the most part to alfalfa or alfalfa-grass mixtures. Because of the successful experience in storing low-moisture alfalfa in a bunker silo, it was decided to investigate the possibility of extending the method to grass crops. Twenty-two tons of second cutting orchardgrass with an average moisture content of 45% was stored in a bunker silo. The sides and top surface were sealed with 4 mil polyethylene plastic. A dry matter recovery of 87% appears promising in spite of some mechanical damage by tramping with cattle and puncturing of the plastic cover. Dry matter consumption of the silage was significantly higher than from direct-cut orchardgrass silage stored in a conventional tower silo. (AH h3-3)

3. Effect of nitrogen fertilization and stage of maturity on the quality and feeding value of orchardgrass silage. Nitrogen fertilized and unfertilized orchardgrass was ensiled at two stages of maturity using the direct-cut method. Nitrogen fertilization increased the crude protein content of both forage and silage, reduced silage consumption and liveweight gains at both early and late maturity stages. The 10-day delay in harvest time reduced dry matter digestibility about 7 units in the nonfertilized silage, but only 2 units in the nitrogen fertilized silage. Within cow variations in silage dry matter consumption were largely explainable (63%) by a multiple regression using four factors, namely, dry matter percent, butyric acid percent, lactic acid percent, and the ratio of ammonia nitrogen to total nitrogen. It will be noted that the use of nitrogen fertilizers on orchardgrass, while increasing yield of dry matter per acre, increases the difficulty of making a good quality of silage and is one of the reasons for present confusion on proper methods for harvesting and processing the crops as silage. (AH h3-3)

4. Methods of improving the quality of orchardgrass silage produced from nitrogen fertilized grass. Previous experiments have shown that direct-cut orchardgrass silage produced from heavy nitrogen fertilization was generally of poor quality and the intake by cattle reduced. Addition of sugar to provide an initial sugar to protein ratio of 0.5 was somewhat helpful as was the use of a flail cutter which causes some bruising of the forage. Wilting the crop to about 37 and 45% dry matter was more effective. Limited intake data indicated that wilting was more effective than the addition of sugar on increasing dry matter consumption. It has generally been thought that silage reaches a stable condition after 30 days of fermentation. Borings taken 62 days after ensiling showed a marked loss of lactic acid and a concomitant gain in acetic acid and ammoniacal nitrogen. (AH h3-3)

5. Biochemical studies relating to silage investigations. Accumulation of nitrate occurs in some forage crops which have been heavily fertilized with nitrogen and produced under certain conditions. Because the nitrate is toxic to animals and the nitrous oxide fumes from the silo can cause deaths if inhaled, chemical studies were conducted using ground orchardgrass forage in quart jar silos utilizing various treatments. In the first cutting

of orchardgrass, inoculation with silage juice or heating and inoculation, resulted in appreciably elevated pH and also produced sizable reductions in nitrate content. In the second and third cutting spontaneous removal of nitrate occurred without any treatment other than grinding. Neither sulfur, urea, inoculation, heating to 80°C and inoculation, sodium thiosulphate nor iodine appeared to accelerate the conversion.

Evolution of gases from the silages was reduced in first cutting by inoculation and/or heating. Since nitrate reduction also occurred with a rise in pH, complete reduction to NH_4 and retention in the silage is suggested. (AH h3-1)

6. Efficiency of a bunker silo for storage of corn silage. One hundred and twelve tons of corn were cut and stored in a bunker silo and sealed with a weighted 4 mil polyethylene plastic cover. After 3 months storage, the silo was opened and fed for 4 months during the Winter. 91.8% of the stored dry matter was recovered as feedable silage. This experiment indicates, along with previously collected data, that bunker silos can be managed in such a way that 90% of the stored corn crop can be recovered as good silage. In view of the generally low initial cost of the bunker and the ease of filling, they appear to have an important place in economical storage of corn silage. (AH h3-3)

7. Use of fungicides for reduction of surface spoilage in low-moisture alfalfa silage. Surface spoilage of low-moisture silage is a problem of some concern where this method is used to preserve forage crops. Two fungicides, Crag Mylon (3-5 dimethyltetra hydro -1, 3-5, 2H - thiadiazine -2-thione) and para formaldehyde were studied in 4 x 8 silos for their effectiveness in preventing surface spoilage. Both materials produced promising results, but inconclusive evidence for reducing top spoilage. (AH h3-3)

8. Comparison of RS-610 grain sorghum and star pearl millet as silage with the utilization of the regrowth for late summer soiling. Studies conducted at Lewisburg, Tennessee, showed that RS-610 grain sorghum produced more dry matter per acre than Starr Pearl millet (9871 vs 7602 lbs per acre). On the other hand, intake of dry matter and milk production per cow was significantly higher for the millet. However, the grain sorghum regrowth produced slightly more milk when fed as green chop. (AH h3-12)

9. Stored forage compared to rotational grazing for lactation. A study has been in progress at Lewisburg, Tennessee, comparing stored forage systems with rotational grazing for lactating dairy cows. During the fourth year of the experiment, the cows on pasture produced slightly more milk than those fed stored forage (45.0 vs 43.1 FCM per day). On the other hand, carrying capacity in days for each 8 acre field was 1645 vs 3010 days. The plant population in percent were alfalfa 8.1 vs 48.9, orchardgrass 51.6 vs 29.7, Ladino clover 21.0 vs 16.2, weeds 19.3 vs 5.2. Thus there would appear to be considerable advantage to the stored forage system in terms of milk per acre and persistence of the pasture stand. (AH h3-12)

C. Heifer Feeding

1. Ammoniacal nitrogen relationships as affecting silage intake.

Studies have continued in an attempt to determine why dry matter consumption by cattle of high moisture grass and/or legume silage (65% moisture and above) is less than for barn-dried hay, cut from the same field at the same time. The storage of grass and/or legume forages as high-moisture silages changes the distribution of the nitrogen fraction into various forms. A larger proportion of the total nitrogen appears as ammoniacal nitrogen compared to hay. One physiological mechanism, by which ammonia might limit consumption, is by absorption of excess ammonia from the rumen into the blood.

Experimental silages were produced by adding sugar at the time of ensiling to inhibit ammonia production. Urea, a good source of ammoniacal nitrogen when metabolized in the rumen, was added to other silages before feeding. These two additions, sugar before ensiling and urea after ensiling, supplied the animals with forages in which the naturally occurring simultaneous changes in chemical content were shifted from their normal relationship.

Analyses performed for rumen ammonia showed that it reached about one-half that usually shown to exceed the conversion threshold of the liver which would result in an increased level of blood ammonia.

Other experiments comparing the rumen ammonia level while feeding a hay ration compared to a silage ration have shown no difference in rumen ammonia level. The feeding of urea to 2 animals, receiving hay and 2 animals receiving hay and grain (50-50) in amounts of 100, 200 and 300 grams per day, did not cause a progressive increase in rumen ammonia levels. In fact, the animals seem to be able to adapt themselves to these intakes. Dry matter intake was only slightly reduced at the high intake of urea. These results indicate that the ammoniacal nitrogen level in silage does not act to reduce intake by increased absorption of ammonia from the rumen. However, there remains a second possibility that the ammoniacal nitrogen level in silage may limit feed intake by an effect on taste of the silage. (AH hl-1)

2. Histamine relationships. It has been suggested that the histamine content of silage might affect intake and it has been reported that the histamine content of the urine of silage-fed animals is high. Silages and hay fed to dairy heifers, and the fecal and urinary samples collected from these animals, were analyzed for free histamine. In the silages analyzed, the correlation between the logarithm of the histamine concentrations and the dry matter intake were low, although the correlation between the logarithm of the histamine concentrations and the dry matter content of the silages was high.

Hay and urine of hay-fed heifers were relatively low in histamine. In haylage and silage-fed heifers, high urinary and fecal histamine levels

were not necessarily associated with high histamine intake. The addition of 5 gm histamine-dihydrochloride, to an animal consuming 2.1 lb of chopped alfalfa hay per 100 lb body weight, did not decrease dry matter intake. The data suggest that there is no relation between silage dry matter intake and histamine concentration of the silage. (AH h1-1)

D. Management Practices, Equipment and Facilities

1. Bovine mastitis. Body cell counts were made on 3,872 quarter milk samples from the Beltsville herd and 492 quarter samples from two other herds. These quarters were determined to be free from bacterial infection and normal in all respects. The number of body cells per ml of fore milk ranged from less than 30,000 to more than 8,000,000. Even though a majority of these non-infected quarter samples contained less than 500,000 cells per ml, about 25% of them had counts exceeding 1,000,000. Preliminary data indicate that time of day the quarter samples are drawn has a large influence on the cell count. Samples taken a few hours after milking have higher counts than those drawn a short time before milking.

Two commercial milking machines are being tested on contemporary groups of cows. One machine operates at 15" HG vacuum, has a pulsation rate of 60 per minute and a pulsation ratio of 2-1/2:1. Statistics for the other machine are 12-1/2" Hg vacuum, 48 pulsations per minute and a 1:1 pulsation ratio. The machine with the higher vacuum, faster rate and wide pulsation ratio milks faster during the maximum rate of flow and thus requires less machine time until the milk ejection drops to 1 lb per minute. However, more stripping time is required than for the other machine. The data indicate a machine-cow interaction, especially with cows that are slow milkers. The total milking time per cow ranges from 2.8 to 10 minutes. The average for all cows was 5.5 minutes with no difference between machines. To date, no significant differences between machines have occurred in incidence of mastitis, new udder infections, or number of body cells shed. The high vacuum machine has caused some teat-end erosion. (AH g3-8)

2. Relation of rate of cooling to milk quality. Investigations were begun to determine the effect on milk quality of varying the rate of cooling provided in mechanically refrigerated farm bulk milk tanks. From a series of experiments with an ice-bank tank, it appears that when the quality of incoming milk is high (standard plate count of 10,000 to 40,000) the cooling rate can be decreased considerably below the maximum capacity of the refrigeration system without resulting in an increase in the microflora. The operating conditions leading to minimum detectable bacterial multiplication lay between a five-minute and ten-minute cycle with one-half minute cooling water circulation. These two cycles cooled the first milking of the trial at rate of approximately 75 and 48 BRU/hr/gal from the end of milking to 50°F, respectively. From the beginning of milking, the milk was cooled to 50°F in about 4 and 6 hours. In the latter situation, the bacterial population rose to 8×10^6 per ml by the end of the 48-hour period. In the range studied, changes in cooling rate did not affect either the flavor score of the milk or the acid degree value.

The relation between the temperature history of the milk and the bacterial growth rate was not constant. An initial lag, followed in the second cooling and storage period by temperature-dependent multiplication, was expected. In the period following the third milking, however, there was an actual decrease in the bacterial population. The magnitude of this decrease varied directly with the population. During cooling and storage following the fourth milking, the bacterial population again increased at a temperature-dependent rate. This anomalous pattern of growth will be further investigated. (AH g3-10)

3. Physical methods for fly control. Tests of the effectiveness of various commercial lamps in attracting flies to electrocutor grids were conducted in outdoor cages. Face flies were the most effectively attracted of three species tested and responded to daylight, blacklight BL and blacklight BLB lamps; however, this has only proved successful with confined populations, and attempts to kill face flies around barns with similar traps have been ineffective. House flies also were attracted by daylight, blacklight BL and blacklight BLB lamps, the percentage of a confined population attracted usually being 40-65%. Stable flies were less attracted to light than either face flies or house flies. Observations indicated that the blood feeding schedule prior to testing greatly affects their behavior.

Suitable procedures were developed for testing the reactions of face flies to monochromatic light in a Y-chamber. Initial trials indicate greatest attraction in the blacklight ultra-violet region with progressively reduced attraction at both longer and shorter wavelengths.

Biological studies of both stable flies and face flies were conducted in an effort to determine characteristics which might be capitalized upon in devising control measures and to learn more about the flies' reproductive cycles. Successful matings of stable flies occurred between one-day-old males and five-day-old females and between one-day-old females and five-day-old males; however, a greater proportion of successful matings were indicated when both males and females were five days old. Oviposition studies indicate females begin laying eggs when about 8 days old and may lay as many as 600 eggs in their lifetime, which may be as long as 4-6 weeks.

Contrary to previous information, no significant differences could be found after four generations among colonies of face flies reared under 5, 50 or 500 ft.-candles of illumination, simplifying colony room requirements. Female face flies can lay fertile eggs within 4 days after emergence, deposit their eggs in batches of about 20 eggs per batch at intervals of 2-8 days, and are capable of depositing eggs throughout their lifetime. One female which did not have an opportunity to mate after she was four days old lived 58 days and laid 230 eggs in 10 batches from which 189 pupae were collected.

Approximately 24,000 individually marked face flies were released in an effort to study the flies' dispersal. Marked flies were found two miles from the

release point 24 hours after release and 4 miles away after five days. Attempts to locate face flies in barns at night during the summer have been completely negative. Marked face flies have been observed resting in trees at night. (AH g3-12)

4. Evaluation of fans, sprinklers and shade to alleviate summer temperature conditions for lactating cows. Two years of work has shown that providing fans and sprinklers for lactating Jersey cows during the summer months at Tifton, Georgia, had little effect on cow performance as compared to effect of shade alone. Average daily roughage dry matter intake per cwt. was 1.81 pounds for cows with shade plus fans and sprinklers as compared to 1.76 pounds for the shade treatment. Cows on the shade treatment had an average daily decline in milk yield of 0.185 lb as compared to 0.196 lb for cows on the shade plus fans and sprinklers. Body weight changes of cows on the two treatments were not different. The average A.M. and P.M. rectal temperature was 101.7 and 102.4°F for the shaded cows and 101.9 and 102.2°F for the shaded, fanned and sprinkled cows. Previous work had shown that provision of shades had little effect on summer production of dairy cat cattle in this environment.

The proper height of artificial shades for cattle in the southeast was studied during the summer for three years at Tifton, Georgia. Temperature and radiation at animal height was consistently less under the 6 foot shades than under either 9 or 12 foot shades. (AH g4-3)

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DAIRY PERFORMANCE AND MANAGEMENT RECORDS
Animal Husbandry Research Division, ARS

Problem. Livestock and poultry improvement cannot be accomplished effectively without adequate records of performance and management. Furthermore, records which have widespread utility must be produced through carefully coordinated programs in order that uniformity may be obtained in measurements and analytical procedures. Continual revision of record procurement and evaluation techniques in accordance with current research findings requires integration of program operations and research. Only in this way can there be a continual chain of discovery, application, and field testing.

USDA AND COOPERATIVE PROGRAM

This is a continuing long-term program of performance testing dairy cattle and poultry, including the evaluation of the genetic merit of dairy cows, sires and herds, chickens for egg or meat production, and turkeys. Also included in the program is the control of hatchery disseminated poultry diseases. The work on dairy cattle performance testing is cooperative with 50 States and Puerto Rico and the Records and Breeding Committees of the American Dairy Science Association. Cooperation is also carried out with the National Association of Artificial Breeders and the various dairy cattle breed registry organizations. The poultry work is cooperative with Official State Agencies in 47 States and with the supervisors of 23 random sample tests in the United States and Canada.

The Federal scientific effort devoted to the programs in this area totals 6.5 professional man-years. Of this number, dairy cattle work accounts for 3.3, 3.0 devoted to performance testing and 0.3 to program leadership, and poultry work accounts for 3.2, 3.0 devoted to performance testing, and 0.2 to program leadership.

PROGRAM OF STATE EXPERIMENT STATIONS

The effort of the State stations in this area is quite large. It is difficult, however, to make a manpower estimate which would be distinct from that in breeding sections. As the USDA material indicates, much of the effort in DHIA and in poultry testing is cooperative with the States. At several State stations, DHIA records are processed for dairymen on a reimbursable basis. Data derived is used in estimation of genetic parameters, etc., by resident investigators. Similarly, the random sample poultry tests on broiler and egg production strains conducted at State locations provide information on performance and mortality of commercial stocks. Eggs and meat from these poultry tests are used in detailed studies such as strain comparison of chemical constituents and correlation of these items with production traits.

Animal performance and management records with beef cattle, swine, and sheep are frequently obtained through cooperation with producers, extension service, and industry. In several States, swine testing stations have been established and operated on a self-sufficient basis supported by fees. Research personnel often act in an advisory capacity. In many States, programs of on-the-farm performance and progeny testing of beef cattle have been developed. Again, research and extension people frequently cooperate in this endeavor. At central facilities in some States, bulls belonging to producers are tested for growth and feed efficiency often under the direction of research personnel. Similarly, a limited number of ram testing stations have been developed for indicating growth rate.

No estimate of State station professional man-years is made.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

Dairy Cattle

1. Sire evaluation program. Using 1,911,102 records of performance which were reported during the year, along with the total of 10 million records available in magnetic tape files, 22,313 genetic appraisals of sires were made and the industry was provided 66,383 individual sire records. These genetic appraisals were made on a quarterly basis and included all sires qualifying with five or more progeny having herdmates. A total of 1,338,130 progeny were included in the genetic appraisals. (AH i4-1)

2. Cow evaluation program. Genetic appraisals of dairy cows were performed initially during the year and according to the index,

$$I = b_1(C - H_c) + b_2(S - H_s),$$

where b_1 is the appropriate weight for the cow, C is the cow's average performance, H_c is the adjusted average of the cow's herdmates, b_2 is the appropriate weight for the sire, S is the average of the sire's paternal half sibs, and H_s is the adjusted average of the herdmates of the paternal half sibs. This indexing procedure was developed and implemented in order to provide the industry with reliable estimates of breeding value of the cows enrolled in dairy recordkeeping. Approximately 500,000 cows, which represent registered progeny of AI bulls, were appraised and the top 2% appropriately identified for use by the industry. (AH i4-1)

3. Dairy recordkeeping programs. The 1,420 dairy herd improvement associations employing 2,474 supervisors provide the organizational machinery for operating the program in the 50 cooperating States. Participation in the National Cooperative Dairy Herd Improvement Program continued to expand and was as follows:

<u>Plans</u>	<u>Herds</u>	<u>Cows</u>
Standard DHIA	40,670	2,010,144
Owner-Sampler	25,598	752,229
Weigh-a-Day-a-Month	<u>1,396</u>	<u>60,149</u>
Total	67,664	2,822,522

A total of 1,911,102 records of performance were reported to the Dairy Cattle Research Branch for use in genetic appraisal evaluations and research.

The artificial insemination program, through which the superior sires developed and recognized in DHIA herds are utilized, bred a total of 7,438,293 dairy and 235,289 beef cows. This represents 41.2% of the nation's dairy cows of breeding age. (AH i4-2)

4. DHIA record analysis and research

(a) Recordkeeping statistics. Cows in standard DHIA herds produced 11,286 pounds of milk and 434 pounds of fat in 1962-63, and exceeded cows not enrolled in recordkeeping by 4,136 pounds of milk. Cows were fed an average of 3,900 pounds of concentrates, produced a product valued at \$495, and returned to the farmer an income over feed cost of \$277 per cow. (AH i4-3)

(b) Regression of a sire's breeding value. Theoretical regressions of the breeding value of sires on daughter average, average deviations from herd mates, and adjusted daughter averages were derived. It was concluded that adjustment for variation in the number of herd mates is necessary and that use of the adjusted herd mate average, as currently performed in the USDA sire evaluation program, achieves this end. (AH i4-3)

(c) Biases in adjustment factors for age. Gross and paired methods were used to compute age conversion factors for each of four geographic regions of the United States. Only one region (Western Midwest) showed a serious discrepancy between the two sets of factors. (AH i4-3)

(d) Value of information on mates of sires in artificial insemination (AI). Estimates of breeding value of 207 AI sires were compared, based on daughter deviations and daughter deviations plus dam deviations. The rank correlation between the two estimates was +0.998, indicating that information from dams does not increase the accuracy of sire evaluations. Heritability estimate for milk yield, based on daughter-dam regression, was 0.24. (AH i4-3)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Dairy Cattle

DHIA Sire Summary List. Sept. 1963. ARS-44-132, 364 pp.; Nov. 1963. ARS-44-135, 218 pp.; April 1964. ARS-44-140, 509 pp.; June 1964. ARS-44-145, 153 pp.

DHIA Cow Index List. April 1964. ARS-44-139, 84 pp.

DHIA Cow Performance Index List. June 1964. ARS-44-146, 160 pp.

DHIA Lactation Averages. May 1964. ARS-44-143, 121 pp.

Dairy Herd Improvement Letters. 1963. ARS-44-131, 134, and 136; 1964. ARS-44-137, 138, 141, 144, and 147.

Miller, R. H. 1964. Regression of a sire's breeding value on various functions of daughters' and herdmate production. J. Dairy Sci. 47:305.

PRODUCTION INFLUENCES ON DAIRY STEERS
Animal Husbandry Research Division, ARS

Problem. Beef, lamb, pork, and poultry are excellent sources of wholesome and digestible animal proteins and fatty acids necessary in maintaining a healthy, appetizing diet. However, these meats must be of high quality, as well as in plentiful supply, if they are to retain their high position and esteem in the minds of consumers. Proper finish, a high proportion of lean, with adequate intramuscular fat, tenderness, full flavor, and color desired by the consumer are the goals the meat producer must strive to attain through breeding, feeding, and management. The quality of cuts and kind of meat are directly reflected in the demand and in the price of the product.

Egg shell strength and yolk quality, strength of wool, fatness, quantity, flavor, color, and tenderness of meat are all known to be influenced by production practices. However, these quality characteristics and many more are not well understood, even though they are of considerable economic importance. Effective measures of evaluating quality differences are of great importance in determining the nature and effect of production practices on the products.

USDA AND COOPERATIVE PROGRAM

This is a continuing program conducted by food product technologists, wool and fiber technologists, biochemists, chemists, physiologists, statisticians, and animal husbandmen engaged in both basic and applied research designed to develop methods and information which will be useful in evaluating quality and quantity of animal products and will be useful in aiding and directing livestock production. Research on beef, veal, lamb, and pork is directed at the influence of selection and breeding, nutrition, physiology, management, and other production variables on carcass and meat quality and quantity. Standards are being applied and adapted for appraisal of slaughter animals, of carcasses, and of meat cuts. The objective of the work with poultry and eggs is to ascertain those factors of nutrition, breeding, and management which contribute to the initial quality of poultry products and their capacity to retain that quality. Studies with wool, fur, and fiber are conducted to determine the physical, chemical, and biological structures and properties of wool and other animal fibers as influenced by production factors. Research on humane slaughter was continued on a reduced scale, primarily to bring to a conclusion some phases of electrical immobilization and physiological responses. The work is conducted at Beltsville, Maryland; Dubois, Idaho; Fort Wingate, New Mexico; and in cooperation with eight State experiment stations. Cooperation is also carried out with the Eastern and Western Utilization Research and Development Divisions, the Human Nutrition Research Division, the Agricultural Engineering Research Division, and the Market Quality Research Division.

PROGRESS - USDA AND COOPERATIVE PROGRAMS

Quality and quantity of meat as affected by production 1/

Beef from beef, dual-purpose, and dairy type steers. Holstein steers had the largest rib eyes (11.4 square inches) and the Jersey the smallest (8.9 sq. in.). However, when expressed in inches per hundred pounds carcass weight, the breeds representing the three types ranked as follows: Angus, Holstein, Jersey, and dual-purpose Shorthorn with values of 1.95, 1.82, 1.75, and 1.61 square inches, respectively. Feeding only hay to a portion of the steers resulted in less marbling of the loin eye and in a smaller total rib-eye area. However, expressed as area/cwt. carcass, the values were 1.88 inches compared with 1.80 for the other two rations. A ratio of lean meat to bone showed the Hereford steers to have the largest ratio, 3.69:1; followed by dual-purpose Shorthorns and Angus 3.60:1; Holsteins 2.97:1; and Jerseys 2.84:1. Beef-type steers had more extensive marbling deposits in the lean than did animals of the other types and higher slaughter and carcass grades. The palatability panel noted no breed differences in the 9-11 rib roast sample in desirability of aroma, but the flavor of lean for the Angus was one-half panel-unit higher than for the Jersey. No significant palatability differences were noted among 9-11 rib roast samples for the non-beef type steers for tenderness, juiciness, and overall desirability. Beef type steers rated slightly higher in these three categories than non-beef type steers. There were continued significant differences in eating quality of 9-11 rib roast samples from calves fed milk and calves fed milk replacer. Samples from calves fed milk were significantly more tender at six months of age than those fed replacer. However, when similar calves were fed to slaughter weight on concentrates or hay, they had recovered from any deleterious effects due to feeding the first six months on milk replacer. (AH d3-6)

PUBLICATIONS - USDA AND COOPERATIVE PROGRAMS

Bond, James; Hooven, N. W.; Thornton, J. W.; Hiner, R. L.; and Warwick, E. J. 1963. Influence of breed and plane of nutrition on beef production from dairy, dual-purpose, and beef steers. (Symposium on Beef Production from Dairy Cattle, Rome, Italy, August 1963) (AH d3-6 and AH d4-7)

1/ See also page 5.

INFECTIOUS AND NON-INFECTIOUS DISEASES OF CATTLE
Animal Disease and Parasite Research Division, ARS

Problem. Losses from infectious and non-infectious diseases of cattle, other than those due to parasites, are estimated at approximately \$600 million annually. These losses materially increase costs of production and conversely decrease profits. In turn, they contribute to the cost of every purchase of meat, milk, and other cattle products to the consumer. Some of these diseases are transmissible to man. Determination and definition of the causes of cattle diseases, explorations for efficient methods of diagnosis, prevention, control, and when feasible, eradication, are the purposes of the research program.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of cattle. Research is being conducted on the diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 57.7 professional man-years. This effort is divided among sub-headings as follows:

Brucellosis of Cattle 2.3 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the University of Minnesota, the University of Wisconsin, and with the Ohio Agricultural Experiment Station. A project on the immunizing effect of Brucella cell wall is in progress at the Hebrew University, Jerusalem, Israel, under a PL 480 Grant of funds equivalent to \$31,950.00 over a 3-year period.

Vibriosis of Cattle 5.1 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the New York State Veterinary College at Ithaca.

Tuberculosis of Cattle 6.6 at the National Animal Disease Laboratory, Ames, Iowa, and through two contracts with the Michigan State University at East Lansing.

Mucosal-Respiratory Disease-Complex 5.1 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Colorado State University at Fort Collins, the Agricultural Experiment Station, Purdue University at Lafayette, Indiana, and the Iowa State University, Ames.

Mastitis of Cattle 6.2 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California, Davis.

Respiratory Disease of Cattle (Shipping Fever) 5.0 at the National Animal Disease Laboratory, Ames, Iowa.

Leptospirosis of Cattle 6.0 at the National Animal Disease Laboratory, Ames, Iowa.

Infertility in Cattle, other than Vibriosis and Trichomoniasis 3.0 at the National Animal Disease Laboratory, Ames, Iowa.

Epizootic Bovine Abortion 3.4 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the Agricultural Experiment Station at Ames.

Foot Rot (Infectious Pododermatitis) of Cattle 4.0 at the National Animal Disease Laboratory, Ames, Iowa.

Etiological, Cytological and Histochemical Studies of Pulmonary Adenomatosis in Cattle 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Immunization Against Bovine Leptospirosis 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Chemotherapy in Leptospirosis 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Nature and Immunogenicity of Leptospiral Lipids 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Paratuberculosis of Cattle (Johne's Disease) 5.0 at the National Animal Disease Laboratory, Ames, Iowa.

Keratitis (Pink Eye) 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

PROGRAM OF STATE EXPERIMENT STATIONS

The State experiment stations are active in conducting basic and applied research pertaining to the prevention, control and eradication of diseases of cattle. Objectives of these studies not only concern the health and well-being of animals but also reflect the increasing interest in the role of diseases of animals to the health of human beings. Research workers are concerned in delineating the cause of specific conditions, developing techniques for the improvement of diagnoses, finding new methods of increasing resistance to disease and/or decreasing the exposure to infectious agents.

Factors which affect the immune response in vaccinated calves and the development of new tests to increase the speed and accuracy by which brucellosis-infected animals can be detected are under investigation.

Cooperative regional studies among the Northeastern (NE-40, Pathology of Breeding Failure) and Southern States (S-30, Diseases of Reproduction) seek to determine the relation of infectious agents to poor reproductive performance and sterility in cattle. Antigenic variations in strains of the organism causing vibriosis are being studied to improve diagnostic techniques and to develop possible immunizing agents. The role of leptospira in infertility is being determined and detailed studies on the pathology produced by different serotypes of the organism are being elucidated.

Many of the North Central States are cooperating informally (NCR-37), Mucosal Disease; NCR-29, Shipping Fever) to determine the causes of bovine respiratory problems and to develop methods for control. Preventive vaccines are being developed and evaluated under laboratory and field conditions. The relation of infectious bovine rhinotracheitis to the respiratory disease complex is also being investigated.

Studies seek basic information pertaining to the cause of mastitis and the fundamental factors that influence resistance of individual cows. Prophylactic and therapeutic agents are being studied to evaluate their efficacy and milk residue properties.

Workers in many States are studying the interrelationships between various agents and factors associated with intestinal infections in cattle, particularly those causing severe losses in newborn calves.

Attempts are being made to clarify the cause of foot rot and infectious keratitis or pink eye. There is some evidence that viral agents may be responsible for these conditions.

Much attention (Regional Research Project, W-41, Urinary Calculi of Beef Cattle) is being given to possible factors which lead to the development of urinary calculi of cattle. Consideration is being given to the theory that an imbalance of certain nutritional elements may contribute to the development of the condition.

New diseases are being encountered constantly and diseases not previously encountered or not regarded as a problem, often become economically important enough to require intensive study. Other bovine disease problems being investigated currently include the various abnormalities, malignant lymphoma, tuberculosis, paratuberculosis, epizootic abortion, ketosis, parturient paresis, white muscle disease, aplastic anemia, enterotoxemia, etc.

The total State scientific effort devoted to diseases of cattle is 52.8 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Brucellosis of Cattle

Research work conducted at the National Animal Disease Laboratory (NADL), Ames, Iowa, was reported as follows:

1. Pathology: a) Two bulls, naturally infected with Brucella abortus were studied for 5 and 2 years, respectively. Serologic, bacteriologic and histopathologic examinations were correlated with the clinical signs of the disease. Seroagglutinin and semen plasma agglutinin titers persisted at diagnostic levels throughout the study, and Brucella abortus was consistently isolated from the semen of both bulls. At necropsy Brucella abortus was isolated from the testes, epididymides, seminal vesicles and the ampullae of the ductus deferens. Pathologic changes were observed throughout the genital tract. Granulomas, including sperm granulomas, were found in the epididymis of one bull.

b) In two other bulls infected with brucellosis, the etiologic agent was Brucella abortus Strain 19. One bull, vaccinated at 6 months of age developed a bilateral orchitis within 10 days. Two months postvaccination a bilateral castration was required. A second bull was vaccinated at 5 months of age. Eight months later the seroagglutination test showed the bull had a titer of +50. The semen plasma test titer was +400. Brucella abortus Strain 19 was readily isolated from the ejaculate of each bull. At necropsy Strain 19 was isolated from the seminal vesicles, prostate, urethra and the epididymides. Pathologic alterations were primarily confined to the accessory genital organs and semen quality was not noticeably affected. The necessity and wisdom of employing the semen plasma agglutination test in addition to the blood serum tests was clearly indicated as a means of detecting potential spreaders of brucellosis.

2. Serology: a) Nonspecific agglutinins for Brucella were isolated from 7 cattle by techniques for absorption of serum by Brucella cells and differential ultracentrifugation. The agglutinins had high molecular weights with sedimentation coefficients varying from 13.8 to 16.6 Svedburg units. The isoelectric points of the purified agglutinins determined by microelectrophoresis correlated positively with the heat stability of the seroagglutinins. With one exception the heat stable (56 C, 18 hr.) agglutinins had an isoelectric point of pH 4.7, whereas the heat labile agglutinins had an isoelectric point of pH 4.3. The activity of the purified agglutinins ranged from 0.1 to 0.5 ug of protein per unit of agglutinin. The ultraviolet absorption maximums of the agglutinins fell into the range of typical proteins.

b) These physicochemical studies were continued on Brucella agglutinins produced by heifers after vaccination with Brucella abortus Strain 19. One week after vaccination all agglutinins were of high molecular weight. In the second week postvaccination some low molecular weight seroagglutinins

were detected. The high molecular weight agglutinins reached a maximum concentration approximately 13 days postvaccination. Low molecular weight agglutinins reached a maximum at 28 to 42 days postvaccination. With few exceptions the fast sedimenting agglutinins predominated throughout the 91-day study. There was a positive correlation between the percentage of heat labile (65 C, 15 minutes) agglutinins and the percentage of fast sedimenting agglutinins. The percentage of agglutinins inactivated by mercaptoethanol was closely related to the percentage of fast sedimenting agglutinins.

c) A continuation of this research involved density gradient ultracentrifugation and heat stability (65 C, 15 minutes) studies on *Brucella* seroagglutinins of pregnant heifers artificially infected with virulent *Brucella abortus*. During the first two weeks postexposure, all of the agglutinins detected were high molecular weight type. Low molecular weight agglutinins were first detected between the 15th and 29th days postexposure. As infection progressed, the concentration of slow sedimenting (low molecular weight) agglutinins became equal to and then exceeded that of the high molecular weight agglutinins. As in the former studies there was a high positive correlation between the percentage of heat labile agglutinins and the percentage of high molecular weight agglutinins in each serum.

3. Immunology

Eighteen vaccinated and 5 nonvaccinated heifers in midgestation were exposed to virulent *Brucella abortus* strain 2308. Changes in their serum proteins were studied by paper electrophoresis for 29 weeks postexposure. In the serums of the heifers that became infected (4 vaccinated and 4 nonvaccinated), the relative percentage of gamma globulin over albumin was greater and persisted longer in the serums from infected nonvaccinated than in infected vaccinated heifers. Changes in the amount of gamma globulin roughly paralleled the changes in the seroagglutinin titers. Only minor changes occurred in the concentrations of albumin and globulin in the serums from 14 vaccinated and one nonvaccinated heifer that did not become infected.

(Iowa) (ADP al-3(Rev.))

The University of Minnesota, under a cooperative agreement with the USDA, reported the studies during the past year were concerned with the study of physico-chemical characterization of antibodies for *Brucella* found in milk and serum of cattle and swine, and the development of methods to separate the several classes of antibodies for *Brucella* found in bovine milk.

(Minnesota) (ADP al-3(Rev.))

The University of Wisconsin, under a cooperative agreement with the USDA, reported work on a method of standardizing the complement-fixation (CF) test for bovine brucellosis, utilizing the *Brucella* antigen for the standard serum agglutination tube test. With the International Standard for anti-*Brucella abortus* serum, the method compared favorably in sensitivity with methods used in European laboratories.

Over 1400 serum samples from cattle in brucellosis problem herds in Wisconsin were examined by the standard serum agglutination tube test and the CF test. The CF test was a useful supplemental test for serums with suspect titers to the agglutination test. In several herds in which infection was recent, cattle developed CF titers before they became agglutination reactors. (Wisconsin) (ADP al-3(Rev.))

Research work was initiated at the Ohio Agricultural Experiment Station, Wooster, under a cooperative agreement with the USDA. The first phase of the study on early vaccination of calves against Brucellosis was completed. Calves 2 and 3-months of age, respectively, were tested by serological techniques and then vaccinated with Brucella abortus, Strain 19. (Ohio) (ADP al-3(Rev.))

Investigations on "The Immunizing Effects of Brucella cell wall" are in progress at the Hebrew University, Jerusalem, Israel, under a PL 480 Grant (A10-ADP-6). The preliminary work, using experimental animals, has been mainly of a confirmatory nature. However, it appears encouraging. (Israel)

B. Vibriosis

Research conducted at the National Animal Disease Laboratory, Ames, Iowa, was reported as follows:

1. Reproductive Patterns of Vibrio fetus-infected Cattle. Work has been completed on a study of the breeding patterns of 31 female cattle bred for 1 to 4 successive calf crops to Vibrio fetus-infected bulls. Twenty-eight of these cows became infected at first service to an infected bull. One of the remaining 3 became infected at first service for her second calf; one became infected at second service to an infected bull, and the other did not become infected when bred for 2 pregnancies.

The average duration of infection with V. fetus was 180 days, ranging from 14 to 313 days. All except 1 cow recovered spontaneously between gestation periods and remained free of infection until they were rebred, at which time 60% became reinfected. The exception was one heifer which remained infected during gestation and thereafter until necropsy, 66 days after calving, when V. fetus was isolated from her uterus.

The cows required more services and more time from first service to pregnancy when first infected than did those which were reinfected; however, some reinfected cows also remained infected throughout subsequent gestation periods.

This study indicates that although immunity was not established with first infection, heifers artificially exposed with V. fetus at sexual maturity might be stimulated to produce resistance before service for their first calf crop and thus breed satisfactorily without significant lost time.

2. Vibrio Infection of the Digestive Organs of Cattle. Eighteen cattle were orally inoculated with broth suspensions of Vibrio fetus type 1, subtype 1, and type 2 to study the infectivity of each for the digestive organs. Six of 7 cattle fed type 2 became infected and shed the organism in their feces for variable periods. One cow remained infected for 4 weeks. She became reinfected after feeding this type again and remained infected for 16 additional weeks. Another cow, infected for 6 weeks, did not become reinfected when inoculated a second time. Neither type 1 nor subtype 1 V. fetus infected any of 11 cows inoculated. At necropsy type 2 V. fetus was isolated from the duodenum, bile, bile duct, liver, and pancreatic duct of cattle up to 5 months after feeding.

It was apparent from this study that only type 2 V. fetus infects the digestive organs of cattle and although type 1 and subtype 1 proliferated in the reproductive organs of cattle and caused repeat breeding, they are unable to live in the digestive organs. Type 2 has been considered an intestinal inhabitant which has the capacity to cause sporadic abortion in cattle and sheep.

3. Fluorescent Antibody Studies. Fluorescent antibody conjugates capable of producing fluorescence in V. fetus cells were prepared by several methods. It appears that the success of fluorescent staining varies with the method of serum fractionation employed. The performance of conjugates prepared from serum fractionated with ammonium sulfate was superior to that of other conjugates. Bright staining was observed more frequently with bovine serum conjugates than with conjugates of rabbit origin. While the staining of cell suspensions was rapid and simple, better results were obtained by staining smears. (Iowa) (ADP al-9(Rev.)

The New York Veterinary College, Cornell University at Ithaca, under a cooperative agreement with the USDA, continued research studies on diagnostic procedures for vibriosis. The following findings were reported:

A. Incidence of vibriosis in an artificial insemination stud. From 1952 to July 1963, 12,644 semen samples from 432 bulls were cultured for Vibrio fetus. Analysis of the data indicated that 20.4 percent of all bulls examined were carriers of V. fetus. There was a highly significant age effect on the incidence of V. fetus. Of 233 bulls under 6 years of age, 4 (1.7 percent) were carriers, whereas 65 (46.7 percent) of 139 bulls 6 years of age or older were carriers.

B. Diagnosis of vibriosis in the bull by use of fluorescent antibody technique. The objective of this project was to develop a fluorescent antibody technique for diagnosing vibriosis in bulls. Although culture techniques for recovering V. fetus from bulls have been improved during the last few years, limited numbers of culture attempts cannot be relied upon for detecting all carrier bulls. The use of virgin heifers as test animals is more accurate, but is too expensive for routine use.

The fluorescent antibody technique has been successfully adapted for diagnostic purposes by using a conjugate purified to eliminate most of the non-specific staining reactions and by concentrating the organisms in samples of sheath scrapings through centrifugation. On the basis of present results, it appears that this is a more sensitive method of diagnosis than the best culture methods and that it probably ranks with the heifer-mating test in its efficiency. (New York) (ADP al-9(Rev.)

C. Tuberculosis

Research was continued at the Michigan State University under two contracts with the USDA. Reports submitted are as follows:

(Contract No. 12-14-100-6852(45)). Lipids extracted by ether-ethanol from 25 strains of mycobacteria were fractionated by absorption chromatography. Infrared spectra of the fractions were recorded. Type-specific lipid compounds were found in the extracts of human, bovine, avian and atypical strains.

Demoycoceronate of phthioceral was found in the lipids of two human strains (H37R_A and H37R_V) and one bovine strain (M. bovis Ravenel), mycoside B was isolated from M. bovis Ravenel but not from M. bovis B.C.G. Other type-specific lipids found were: mycoside A isolated from two photochromogens (P-4 and P-8), mycoside F from M. fortuitum, mycoside C from M. avium and 158 C-O (isolated from a bovine mesenteric lymph node), mycoside D from 71C-O (also from a bovine mesenteric lymph node) and mycoside C_M from strain P-31 and 12 organisms isolated from swine mesenteric lymph nodes and bovine body lymph nodes and Peyer's patches.

(Contract No. 12-14-100-7164(45)). This contract was initiated during the reporting period. Experiments are in progress. Cattle were obtained which were not sensitive to tuberculin by caudal fold and cervical tests. The necessary facilities have been obtained to permit studies on chromatograph. (Michigan)

Contract No. 12-14-100-5786(45), on the Role of Heat-Killed Mycobacteria and Feed Supplements of Animal Origin in Producing Tuberculin Hypersensitivity in Cattle, was completed, and the researchers at the Michigan State University submit the following summary of their findings:

Sixty nonpregnant predominantly Guernsey crossbred heifers were fed one of four different rations for 160 days to determine if any of the rations would induce delayed hypersensitivity in the animals as detected with 0.1cc mammalian tuberculin injected intradermally. The animals were obtained from a herd with no tuberculin reactors and had no detectable response to mammalian or avian tuberculins or johnin when tested in the caudal fold and cervical regions. They were maintained during the study in four isolated groups of 15 each. The control group was fed a ration in which the protein concentrate was soybean oil meal and the mineral concentrate was dicalcium phosphate. The second and third groups were fed the control ration to which daily was added $5 \times 10^{8-9}$ heat-killed (121C moist heat for 30 minutes) Mycobacterium bovis and Mycobacterium avium, respectively. The fourth group was fed a ration in which the protein concentrate was meat and bone scrap and the mineral concentrate steamed bone meal.

Tuberculin tests using 0.1 cc mammalian tuberculin were performed on all animals at three different times. Some were tested at 20, 30 or 40 days, and all were tested at 100 and 160 days following the start of feeding the experimental rations. No animal was classified as a reactor at the official reading time.

(Michigan)

(ADP al-13(Rev.))

D. Mucosal-Respiratory Disease-Complex of Cattle

Research studies were continued at the National Animal Disease Laboratory, Ames, Iowa. Reports submitted showed that calves inoculated with bovine viral diarrhea (BVD) viruses and soluble antigen, the complement-fixing (CF) antibodies appeared before serum-neutralizing (SN) antibodies and remained at high levels throughout the test period. A rapid rise in SN antibodies occurred after challenge with homologous virus with no apparent effect on CF antibody levels.

The CF antibody responses in calves infected with cytopathogenic NADL-MD and noncytopathogenic CG-1220 viruses were similar, whereas SN antibody responses indicated strain specificity by reciprocal cross-neutralization tests.

The CF antibody levels in 5 hog cholera (HC) antisera were assayed, using the soluble antigen of NADL-mucosal diarrhea-bovine virus diarrhea virus. No demonstrable SN antibodies were present in four HC antisera tested against NADL-MD virus, but a significant titer was present in the commercially prepared antiserum.

Virus was reisolated from animals infected with BVD viruses by buffy coat culture technique during 3 weeks' postinoculation, even when significant levels of CF and SN antibodies were present.

Noncytopathogenic (NCP) bovine viral diarrhea disease agents can be detected and titrated in tissue culture systems by a method employing immunofluorescence. Cytopathogenic (CP) and non-CP (NCP) viruses cross-react with fluorescein-conjugated serum globulins produced against either CP or NCP viruses, but the fluorescence is more intense in the homologous serum. Serum neutralization titers of sera against both CP and NCP groups were compared for both groups of viruses, and results of cross reactions were in agreement with results from immunofluorescence tests. (Iowa-NADL)

Colorado State University, Fort Collins, under a cooperative agreement with the USDA, reported that during the past year the serum neutralization titer of the cattle which were kept in the isolation units did not show lowering of titer. There were 3 lots of cattle with 4 animals per lot. One group was injected intratracheally, one intramuscularly, and the third lot served as control. There was no difference of titer between the two infected lots.

Pathological studies of infectious bovine rhinotracheitis in relation to abortion are being conducted using 35 virgin heifers that were negative to serological tests for this disease, brucellosis, and leptospirosis. Progress is being made on this phase of the research. (Colorado)

Research work conducted at Purdue University, Lafayette, Indiana, under a cooperative agreement with the USDA, was a continuation of tissue culture, fluorescent antibody, and serological investigations.

Sporadic cases of the mucosal disease complex continue to occur in Indiana. The apparent incidence of this disease complex has not changed from previous years.

Cytochemical and cytological studies on the growth of Oregon C24_v virus in tissue culture were made. The application of the acridine orange (AO) staining procedure to infected lamb thyroid cultures gave evidence that C24_v virus is of the RNA type. Furthermore, AO and phase microscopic studies suggest that replication takes place in the cytoplasm of infected cells. The data derived from these and other growth studies will be utilized in applying fluorescent antibody procedures for detection of virus diarrhea-mucosal disease agents in clinical specimens and tissue culture systems.

On initial passage C24_v virus was capable of producing cytopathic changes in cultures of bovine and ovine kidney, testicle and thyroid tissues. In an explant-type culture system employing lamb kidney tissue, cytoplasmic inclusion-like lesions were observed. The development of cytoplasmic inclusions and the general cytopathic effects of virus were inhibited by specific immune serum. Further study is needed to determine the specificity of the cytoplasmic lesions observed.

Two virus isolations made from field cases of "mucosal disease" were grown on bovine embryonic kidney and lamb thyroid cells. Serums from Specific-Pathogen-Free calves recovered from experimental infection with the two field isolates neutralized Oregon C24_v in tissue culture tests. The new virus isolates appear to be immunologically and serologically related to other virus diarrhea-mucosal disease viruses.

The specific-pathogen-free (SPF) cattle herd continues to be relatively free of important pathogens. The reproductive efficiency of the herd is normal and about 24 calves will be available for research during the next twelve months. (Indiana)

At the Iowa State University during the past year, research results have pointed to the fact that both viral diarrhea and infectious bovine rhinotracheitis may elicit a clinical and pathological syndrome which is indistinguishable. They have verified this fact by fluorescent antibody staining of viral antigen associated with Herpes-virus-induced lesions. Results further indicate that the entire group of enteroviruses may be excluded from the viral diarrhea problem in cattle, but play an important role in enteric problems of young calves. (Iowa State Univ.)(ADP al-14C(R))

E. Mastitis of Cattle

The research studies at the National Animal Disease Laboratory, Ames, Iowa, pertained to the following:

1. Three cultures of group A hemolytic streptococci have been serially subcultured for an extended period (100 or more serial transfers) in a peptide- and protein-free medium. In 24-48 hours incubation at 37°C, luxuriant growth was obtained with complete removal of 1 percent glucose and quantitative fermentation to lactic acid. Optical densities of cultures were 0.40 - 0.50. An amino acid assay medium, modified by addition of small amounts of glutamine, ammonium acetate and 0.1 M phosphate, pH 7, was used. In this medium high concentrations of glutamic acid or glutamine were required and biotin was stimulatory to growth. Biotin could be partially replaced with NaHCO₃. Maximal growth was obtained with NaHCO₃ when biotin was present and aspartic acid and asparagine were omitted from the medium.

2. Three strains of Streptococcus pyogenes, Richards (type 3), N19 (type 19) and S43 (type 6), after repeated subculturing in a chemically defined medium (100 or more times), were each tested for the group-specific and type-specific antigens by the precipitin test. On three separate tests for each culture, the group A antigen ("c" polysaccharide) was present, but the type-specific antigen (M-protein) was absent. The same strains grown on a chemically defined medium containing reduced ovalbumin showed no loss of M protein. (Iowa - NADL)

The University of California, Davis, under a cooperative agreement with the USDA, reported that the results of several years of investigation indicate that coliform mastitis is a disease of the normal lactating mammary gland. It was concluded that the relative infrequency of occurrence of coliform mastitis, in commercial dairy herds, is due to the presence of a leukocyte barrier in lactating glands of older cows. This leukocyte barrier is in response to the stress on mammary tissues of modern methods of mechanical milking and bacterial infection with common udder pathogens.

To further substantiate the role of the leukocyte in controlling multiplication of coliform bacteria within the udder, an attempt was made during the current year to delay the infiltration of leukocytes into exposed mammary glands. To this end, corticosteroids, both intramammarily and systemically were employed. Selection of corticosteroids for this purpose was based on the claimed ability to inhibit diapedesis of leukocytes into foci of developing inflammation. The corticoid employed was 9 α fluoroprednisolone acetate (Upjohn) at dose levels of 50 mg. to 1,000 mg. per cow. Such doses were greatly in excess of the quantities commonly incorporated in antibiotic preparations for therapeutic treatment of mastitic glands. Administration of the corticoid prior to and simultaneously with coliform bacteria failed to delay or reduce the magnitude of the leukocytic infiltration. Despite the mobilization of circulating neutrophil leukocytes to levels up to 6 times normal following systemic application of the corticoid, the leukocytic activity within the mammary gland exposed to coliform bacteria was not enhanced. Escherichia coli and Aerobacter aerogenes are not considered true pathogenic bacteria. Clinical disease is produced by the release of endotoxin when the bacterial cells of a massive population are destroyed by leukocytic action.

In order to determine if a leukocyte barrier can exist for recognized pathogens of the mastitis complex, attention was directed toward Streptococcus agalactiae. Through natural selection this organism has become an obligatory parasite of the mammary gland. Its potential for establishment of an enduring infection within the mammary gland is well known. Four lactating heifers and 5 older cows, none of which had any previous exposure to Str. agalactiae were available. Pre-existing leukocytic infiltration into mammary quarters were of natural or experimental origin for investigation of the leukocyte barrier. An adequate number of quarters secreting cell-free milk was available for controls. Among 16 glands serving as control and receiving a single exposure to Str. agalactiae at levels of

5 to 600 colony-forming units, infections were established in 6 glands (37%); among 7 control glands exposed 3 times at 12-hour intervals to between 6 and 400 Str. agalactiae, the infection rate was 50 percent. Thus, it was established that small numbers of Str. agalactiae are potentially capable of establishing infection in essentially normal lactating quarters.

Failure of Str. agalactiae to establish itself within an exposed lactating mammary quarter may be in consequence of one or more or a combination of the following:

- 1) Pre-existing leukocytic infiltration at levels serving as a barrier per se to Str. agalactiae multiplication.
- 2) Capability of a gland to infiltrate millions of leukocytes within the first few hours after introduction of Str. agalactiae. Glands previously injured but having returned to low infiltrating cell numbers appear to be uniquely capable of immediate mobilization of large numbers of leukocytes.
- 3) Participation of humoral factors from infection of opposite quarters with Str. agalactiae and, perhaps, other streptococci.

Experiments on the endotoxin of A. aerogenes confirmed a previous hypothesis that the clinical signs of A. aerogenes peracute mastitis were probably referable to the release of endotoxin following lysis of bacteria by the inflammatory exudate. Endotoxin at levels of from 0.2 mg to 20.0 mg produced the same array of signs and symptoms as those seen following unlimited growth of A. aerogenes. The endotoxin had all the characteristics of endotoxin from R variants of gram negative bacteria. Trials in cats and a horse showed cats to be rather resistant to the endotoxin, whereas one horse died within 9 hours following administration of a safe dose calculated from mouse inoculation data.

The probable identity of the protein "X" with paper electrophoretic mobility intermediate between α -lactalbumin and immune globulin that appears in whey following agalactia of mastitis or in early dry cow secretion was confirmed. The protein when isolated by preparative electrophoresis does not have the same mobility in the isolated state but behaves as immune globulin. The isolation procedure is considered to be sufficiently mild that denaturation was not involved.

(California)

(ADP al-15(Rev.))

F. Respiratory Diseases of Cattle (Shipping Fever)

Research investigations conducted at the National Animal Disease Laboratory, Ames, revealed that smooth Pasteurella haemolytica, after rapid growth in statically incubated broth cultures, decreased in numbers rapidly, and were replaced by nonsmooth variants. Upon continued incubation, smooth cells again predominated. The two phenotypes were alike in general biochemical characteristics, but differed in virulence for mice. The presence of non-

smooth cells in mixed cultures limited the growth of smooth cells. The inhibition of smooth cells correlated with the establishment of definite population densities, and the critical factor was limitation of oxygen in the cultural medium. Selective inhibition did not occur in aerated cultures, but was more pronounced in cultures grown under reduced air pressure. Selective death of smooth cells on slant cultures held at 5°C, and preferential growth of nonsmooth cells, plus death of smooth cells at room temperature, accounted for population changes in stored cultures.

Bovine parainfluenza-3 (PIV-3) virus was isolated from nasal mucus of cattle with signs of shipping fever by amniotic inoculation of 14-day-old embryonated hen's eggs. Virus isolated in this manner could not be demonstrated in the amniotic fluid, but after 3 passages in the amnion could usually be recognized by agglutination of guinea pig erythrocytes. Primary virus isolates could, however, be recognized by inoculation of embryonic bovine kidney (EBK) cells. Multiplication of PIV-3 virus in the embryonated hen's egg did not result in death of the embryo.

Virus isolations from diluted specimens suggested that the chick embryo is more susceptible to PIV-3 virus infection than are tissue cultures of EBK cells. Egg inoculation also permitted the selection of PIV-3 virus in the presence of infectious bovine rhinotracheitis virus which was demonstrated in several samples. Attempts to adapt virus isolated in the amnion to the allantoic cavity of younger embryos were not successful. (Iowa-NADL)

(ADP al-17)

G. Epizootic Bovine Abortion

The University of California, under a cooperative agreement with the USDA, reported the following:

1. Studies to be terminated this year indicate conclusively that vaccination with a modified live virus vaccine prepared from an agent (Miyagawanella felis) related to the virus of epizootic bovine abortion (EBA) is ineffective in preventing abortion due to the EBA virus. The current approach to preventive immunization is by means of a vaccine consisting of an attenuated strain of EBA virus, given when heifers are six months of age and repeated just prior to breeding. Attempts to attenuate the virus are currently in progress.
2. In view of indications that EBA is a venereally-transmitted infection, studies designed to determine the validity of this observation are under way. Should this mode of transmission be proved, artificial insemination is regarded as the ultimate solution to the problem of prevention and control.
3. Progress in serological studies relative to the EBA virus has been made.

4. Preliminary findings suggest that abortion due to the virus of infectious bovine rhinotracheitis (IBR) is restricted to those strains of virus which have acquired an enhanced invasiveness for the blood stream of cattle. This property appears to have been acquired fairly recently as studies of early respiratory isolates of the virus indicate that such strains lack this characteristic.

5. The virus of enzootic abortion of ewes (EAE) has been isolated in ewes in California and Oregon. This represents the first isolation of this virus outside the enzootic areas of Montana, Idaho, and Utah. (California)
(ADP al-21)

H. Immunization Against Bovine Leptospirosis

The National Animal Disease Laboratory, Ames, Iowa, reports that Leptospira pomona and 13 other leptospiral serotypes, were subcultured at weekly intervals for 2 years in a medium primarily composed of Oleic Albumin Complex and NH_4Cl . The albumin functioned as a detoxifier of oleic acid and as a source of nitrogen because continuous subculture was possible without adding NH_4Cl , but at a markedly reduced level of growth. Added vitamin B_{12} was required for growth of representative members of each serotype studied. Additions of NaCl stimulated growth.

A commercial complex of bovine albumin and oleic acid (OAC), which replaced whole rabbit serum in leptospiral medium, was fractionated. The growth-supporting function of each fraction was studied, and the fractions were replaced with specific nutrients. Basal medium supplemented with bovine albumin and sodium oleate or Tween 80 supported good growth of 14 leptospiral serotypes through indefinite subcultures with undiminished growth and unaltered antigenicity.

Oleic albumin complex was extracted with ether. The ether extract, when recombined with extracted OAC, supported good growth. Alkalinized oleic acid, sodium oleate, or Tween 80 satisfactorily supplemented several albumins of bovine origin. Adding lipid to spent medium restored its growth-supporting capability. If Tween 80 was used, 0.5% albumin was adequate for cultivation of Leptospira pomona.

Leptospira grippotyphosa was isolated from the urine of a cow 7 days after abortion. The isolant grew poorly in Stuart's liquid medium and Fletcher's semisolid medium. Experimental semisolid and liquid media, containing bovine albumin fraction V and Tween-80, proved valuable as isolation and growth media. Gerbils and hamsters were more susceptible than guinea pigs and white mice to the newly isolated organism. Serological evidence indicates that L. grippotyphosa is widely distributed in Illinois cattle and swine.

(Iowa-NADL) (ADP al-25)

I. Chemotherapy in Leptospirosis

Investigations at the National Animal Disease Laboratory, Ames, Iowa, have determined the effects of certain antibiotics and polylysine on leptospirae in synthetic medium and medium supplemented with rabbit serum. No differences in sensitivity to antibiotics and polylysine were found among cultures of Leptospira pomona, L. canicola, L. autumnalis, and L. grippotyphosa in synthetic medium. All the antibiotics tested were leptospirastatic in low concentrations. Tylocin and erythromycin were effective in the lowest concentration (0.025 µg/ml) and sterilized cultures the quickest (72 to 96 hours); chlortetracycline and oxytetracycline (0.5 µg/ml) prevented multiplication but failed to sterilize cultures in 10 days. Little or no leptospiral immobilization was observed in cultures containing bacteriostatic levels of antibiotics; most antibiotics lysed leptospirae at ten times the leptospirastatic concentration. In Stuart's medium containing 10% rabbit serum, penicillin and oxytetracycline were two and four times less effective than in synthetic medium, respectively. (Iowa-NADL)

(ADP al-26)

J. Nature and Immunogenicity of Leptospiral Lipids

Research workers at the National Animal Disease Laboratory, Ames, Iowa, reported that Leptospira canicola cells were grown in a chemically characterized medium containing Tween 80. Lipid extracted with chloroform:methanol (2:1) from washed, lyophilized cells was equivalent to 16% of the dry cell weight. Approximately 50% of this lipid was present in the phospholipid fraction.

Fatty acids from whole cells were tentatively identified by gas-liquid chromatography of the methyl esters using diethylene glycol succinate and Apiezon L as liquid phases. Unsaturated esters were removed as mercuric acetate adducts.

Of the dialyzable lipid, octadecenoic acid was the major acid accounting for 47% by weight of the fatty acids. Hexadecanoic acid accounted for 19% of the fatty acids. The next largest component (9%) was an unidentified, unsaturated fatty acid with a carbon number of 15.25 on the DEGS polyester column. The other acids listed in descending order of abundance were: an unidentified saturated acid with the same retention volume as a 17-carbon branched-chain acid, hexadecenoic acid, octadecadienoic acid, tetradecenoic acid, an unsaturated acid with a carbon number of 12.75, octadecanoic acid and traces of octanoic, tetradecanoic acid and several other unidentified acids. Acids with retention volumes corresponding to 17 or 19-carbon cyclopropane fatty acids were not noted. (Iowa-NADL) (ADP al-27)

K. Paratuberculosis (Johne's Disease) of Cattle

The National Animal Disease Laboratory, Ames, Iowa, reported that experiments were conducted to find a combination of decontaminant and medium that would be more satisfactory for the primary cultivation of Mycobacterium paratuberculosis. Sodium hydroxide, sodium hypochlorite, phenol, and benzalkonium chloride (Zephiran) were compared as decontaminants, and specimens treated with these agents were cultured on lymph-node-egg-yolk medium and modified Herrold's medium (an egg-yolk-agar medium containing mycobactin). The most satisfactory combination was decontamination with benzalkonium chloride, followed by inoculation onto modified Herrold's medium. This technique allowed the demonstration of Myco. paratuberculosis in tissues in which the organisms were present in such small numbers that they could not be found by microscopic examination.

Blood samples were obtained periodically for complement-fixation tests from all cattle in a herd of 161-195 where Johne's disease has been an economic problem. Selected tissues of all cattle removed from the herd were examined for Mycobacterium paratuberculosis after slaughter.

Observations were made on 93 cattle eliminated from the herd during a 5-year study. Fifty-four cattle had titers of 1:32 or more; 12 of these developed clinical evidence of Johne's disease, and 23, including the aforementioned 12, harbored M. paratuberculosis. Thirteen of the remaining 39 cattle with serum titers of 1:16 or less harbored the bacillus at slaughter, and 3 of these had developed clinical evidence of Johne's disease. Forty cattle were tested 6 times in 2-1/2 years, a total of 240 tests; titers remained constant or changed only 1 dilution in 185 instances. It increased 2 dilutions in 14 instances, 3 dilutions in 6 instances, 4 dilutions in 5 instances, and 5 dilutions in 1 instance. The titer decreased 2 dilutions in 24 instances, 3 dilutions in 4 instances and 5 dilutions in 1 instance. Six calves, a few days to 2 months old, had titers of 1:32. Titers disappeared within 6 months. (Iowa-NADL) (ADP al-35)

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FOOT-AND-MOUTH AND OTHER EXOTIC INFECTIOUS
DISEASES OF CATTLE
Animal Disease and Parasite Research Division, ARS

Problem. The Congress in 1948 authorized establishment of a laboratory in the United States for research on foot-and-mouth and other exotic animal diseases. The law required that the laboratory and related facilities for research and study be located on a coastal island separated from the mainland by deep, navigable waters. Plum Island was selected as the site for the laboratory on July 28, 1952. The Plum Island Animal Disease Laboratory as a U. S. Department of Agriculture venture came into existence on July 1, 1954, and since that time this laboratory has been responsible for protecting the nation's livestock industry against animal diseases of foreign origin. Foot-and-mouth disease has visited the United States on 9 occasions and each time has been eradicated. The last outbreak of foot-and-mouth disease was in 1929. Contagious bovine pleuropneumonia was eradicated in the 1880's and has not recurred since. Success in keeping these exotic animal diseases out of the United States has been due to a number of factors and a continuing vigilance by U. S. Department of Agriculture personnel.

The establishment of the Plum Island Animal Disease Laboratory and its continuing research program on exotic animal diseases has provided a laboratory in the United States where research on animal disease foreign to our soils is carried out. As new information is developed at the laboratory, it is made available to those agencies in the Department responsible for keeping out livestock animal diseases which do not occur in this country. Foot-and-mouth disease is capable of reducing our overall productivity by 25% in areas where it might become established. The disease exists in all large land areas of the world with the exception of Central and North America, Australia, and New Zealand.

Rinderpest, a disease of cattle, continues to be a serious disease problem in Africa and Asia. This disease is capable of killing 90% or more of the cattle exposed to it. Other diseases for which the laboratory is responsible include contagious bovine pleuropneumonia, Rift Valley fever, East Coast fever, and lumpy skin disease. All of these diseases continue to cause severe losses in other parts of the world. The possibilities of entry of these diseases in the United States continues, primarily because of the progressively increasing scope, speed, and extent of modern international transportation. Information developed at the Plum Island Animal Disease Laboratory is applied to the protection of the nation's livestock against foreign animal diseases.

The research continues to develop and maintain a competence for diagnosis of exotic animal diseases. Fundamental research is being conducted on biological, chemical, and physical properties of the infective agents that may be useful in prevention, control, and eradication of these diseases.

USDA AND COOPERATIVE PROGRAM

The Department at its Plum Island Animal Disease Laboratory has a continuing long-term program involving veterinarians, biochemists, biophysicists, microbiologists, and pathologists engaged in basic and applied research in this problem area. All of this research is conducted at the Plum Island Animal Disease Laboratory, Greenport, New York, except for supplemental field studies on foot-and-mouth disease vaccines which is conducted cooperatively in the Netherlands. The Department is also engaged in research under terms of an Interagency Agreement with the Assistance In Development Program, U. S. State Department, in Kenya, on contagious bovine pleuropneumonia.

The Federal scientific effort devoted to research in this area conducted solely at the Plum Island Animal Disease Laboratory, totals 28.5 professional man-years. This effort is divided among sub-headings as follows:

Histopathology -- foot-and-mouth and other exotic diseases 1.0

Fluorescent antibody technique to locate viruses 1.0

Studies on foot-and-mouth disease virus 2.0

Determine mechanism of antibody formation 0.5

Immune response of cattle to types and sub-types of foot-and-mouth disease virus 1.0

Quantity production of foot-and-mouth disease virus 2.0

Microcinematography of cellular reaction of infected cells 0.5

Establishment and characterization of cell lines and cell strains 1.0

Mechanism of the interaction between foot-and-mouth disease virus molecules and host cells 2.0

Genetic biochemistry of foot-and-mouth disease virus 1.0

Effects of chemical and physical environment on foot-and-mouth disease virus 1.0

Bulk Freeze Drying of foot-and-mouth disease virus vaccine and antiserum 1.0

Investigations of Rinderpest in Cattle 2.5

Survival and Transmission of Foot-and-Mouth Disease Virus in Semen 1.0

Identification, purification and chemical and physical characterization of foot-and-mouth disease virus and other exotic animal viruses 2.0

Immuno-chemical investigations of foot-and-mouth disease virus 1.0

Attenuation of representative types of foot-and-mouth disease virus 1.0

Survival and inactivation of foot-and-mouth disease virus in meat and meat by-products 1.0

Biological mechanism of natural resistance and susceptibility to foot-mouth disease virus 1.0

Biological alteration of foot-and-mouth disease virus from continual residence in cell cultures 1.0

Morphological aspects of virus-cell relationships 1.0

Diagnostic and immunizing procedures for contagious bovine pleuropneumonia 3.0

Work was continued under a PL 480 grant to the Instituto Biologica, Sao Paulo, Brazil for a 5-year study of tissue culture of indigenous strains of foot-and-mouth disease virus, and experimental field vaccination.

Under a PL 480 grant to the Ministry of Agriculture, Laboratories of Foot-and Mouth Disease and Tissue Culture, Etlik, Turkey, research is under way on "Studies of Various Indigenous Types of Foot-and-Mouth Disease Virus, and the Production of a Vaccine for the Control of Foot-and-Mouth Disease in Turkey."

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Histopathology -- Foot-and-Mouth and Other Exotic Diseases

It was demonstrated that the initial mucosal and epithelial lesions of both foot-and-mouth disease and vesicular stomatitis consist of circumscribed, degenerated areas of epithelial tissue. The characteristic vesicles develop from these initial lesions; but vesiculation may not and does not always occur. This knowledge is useful in clinical recognition of the two diseases.

A histopathological survey of cattle received at Plum Island during a period of one year revealed a high incidence of sub-clinical (renal) leptospirosis. Clinical recognition of the disease had never been made on the farm where the cattle were raised. The combined microscopical and serological data indicated the probability of infection with more than one serotype. Information obtained was useful in developing concepts of the character of the disease in cattle.

Microscopic pathology of foot-and-mouth disease in pregnant and lactating mice was described.

Lesions in guinea pigs resulting from estrogen contamination of pelleted feed were described. (ADP a8-1(Rev.))

B. Fluorescent Antibody Technique to Locate Viruses

A fluorescent antibody technique for antigens and/or antibodies related to foot-and-mouth disease was developed. Commercial reagents (except the immune sera) were used. Serums from cattle infected with any of the seven types of foot-and-mouth disease (FMD) consistently gave positive or (in only two instances) suspicious fluorescent antibody reactions. Serums from cattle with a lesser degree of immunity than that conferred by frank infection with virulent virus associated with lesion development, gave negative fluorescent antibody reactions. The technique appears to be useful for detecting animals convalescent from foot-and-mouth disease (from one week to as long as 2 years following infection), but did not distinguish between types of infection. (ADP a8-2(Rev.))

C. Studies on Foot-and-Mouth Disease Virus

Foot-and-mouth disease virus (FMDV), type A, strain 119, was produced in baby hamster kidney cell cultures and treated with acetyleneimine (AEI). The AEI-treated preparation was used to prepare a colloidal aluminum gel (Alhydrogel) vaccine and an oil emulsified vaccine. In the animals vaccinated with oil emulsion vaccine, the neutralizing and complement-fixing antibodies were maintained at levels 30 to 200 times greater than in the animals that received the Alhydrogel vaccine and similarly to that found in animals infected with FMDV. The 19S type antibody was difficult to demonstrate in both groups and did not approach the high level produced in animals undergoing actual infection.

The oil emulsified vaccine was superior to the Alhydrogel vaccine in terms of inducing higher levels of antibody which persisted for a relatively longer period of time.

An understanding of the degree and duration of immunity conferred by FMDV as well as a knowledge of practical methods for evaluating immunity is essential to properly plan and implement field vaccination programs. Investigations of this type require the use of large numbers of cattle over long periods of time and can best be carried out in an area where FMD vaccination is routinely practised. Such studies are being pursued in Holland in cooperation with the Netherlands Ministry of Agriculture. A number of herds consisting of approximately 400 cattle vaccinated and held under field conditions, are included in this study. Vaccinated cattle presented for slaughter at the Amsterdam abattoir are also available.

Serum antibody levels against types O and A FMDV remained high for over two years in most cattle which had received two or more annual vaccinations. An average of 80% of the animals which had experienced three or more annual field vaccinations showed resistance 16-48 months later when exposed to virulent FMDV. In general, a good correlation was observed between serum antibody level and resistance to infection, however, indications are that this relationship may vary somewhat depending on the interval of time elapsing between vaccination and exposure to the virus. (ADP a8-8(Rev.)

D. Determine Mechanism of Antibody Formation

The serologic, physical and chemical characteristics of antibodies produced by guinea pigs and cattle following infection with FMDV or inoculated with inactivated virus preparations, were investigated. The first appearing antibodies were macroglobulins having several different characteristics than the late appearing antibody. The time course of appearance of these two antibody types in the intact animal were studied to provide a better basis for subsequent cellular level experimentation. (ADP a8-10(Rev.)

E. Immune Response of Cattle to Types and Sub-Types of Foot-and-Mouth Disease Virus

Antibody formed in guinea pigs against the noninfective component of FMDV is specific for the noninfectious component, possesses no detectable virus neutralizing activity, and with the limited number of strains tested, appears to be type specific.

Assays of virus neutralizing antibody by tissue culture methods consistently resulted in lower values than those obtained when suckling mice were used as the assay system. Factors influencing this difference were the time and temperature of incubation of the antibody-virus mixture and the presence of a "persistent" fraction. (ADP a8-11(Rev.)

F. Quantity Production of Foot-and-Mouth Disease Virus

A simplified method for preparing bovine calf kidney cells for growth on glass has been developed. Yields of 60 ml. of packed cells are routinely obtained from 100 Gm. of cortical tissue. One thousand or more plaque-forming units (PFU) of FMDV per cell were obtained from cell cultures prepared by this method for growth and assay of the virus.

Although dispersed cells were centrifuged to remove trypsin before preparing cultures, satisfactory cultures were prepared from dispersed cells which had not been centrifuged.

Application of information developed during the year on the effect of various chemical and physical factors on cell susceptibility has resulted in preparation of primary bovine calf kidney cells with increased susceptibility to infection with FMDV. These cells were used for plaque assay

of all seven types of FMDV isolated directly from animals. Plaque assay titers were similar to those obtained in adult steers and suckling mice, demonstrating the utility of these cells for use in detection and assay of field samples of FMDV. Increased yields of FMDV from early passage in these cells reduces or eliminates the necessity of adapting FMDV to tissue culture. This finding may have important application to vaccine production.

For a 7-month period considerable effort was diverted to experiments on detection and assay of virus from lymph nodes of cattle inoculated in Argentina with FMDV. (ADP a8-12(Rev.))

G. Microcinematography of Cellular Reaction of Infected Cells

Development of a microcinematographic technique that is practical for use under animal disease quarantine conditions was accomplished at PIADL.

First indications of culture survival after infection with FMDV were observed in connection with work on this project. This information initiated a line of investigation carried on under Line Project ADP a8-30.

Observations were recorded on the cytopathic effect of FMDV and rinderpest on a variety of cultured cells. (ADP a8-13(Rev.))

H. Establishment and Characterization of Cell Lines and Cell Strains

A lamb testis cell line developed at PIADL was used in determining the neutralizing activity of serums containing antibody against mucosal disease virus. Cells of the lamb testis line have withstood freezing and storage at about -70°C for three and one-half years. (ADP a8-14(Rev.))

I. Immuno-Chemical Investigations of Foot-and-Mouth Disease

The physical-chemical characteristics of antibodies produced by guinea pigs and cattle in response to infection with FMDV were investigated. Animals responded with the early appearance of a macroglobulin type antibody (19S) that disappeared by about the 30th day following infection. Within a few days following the appearance of the macroglobulin antibody (19S), antibody of a smaller size (7S) was demonstrated and this antibody persisted at high levels over an extended period of time. Various other serologic, physical and chemical differences were also found for these two types of antibody. Cattle immunized with inactivated virus preparations showed some qualitative and quantitative differences in the time course of appearance of these antibodies when compared to infected animals. Inactivated virus emulsified in oil induced higher antibody levels that persisted for a longer time than when the inactivated virus was adsorbed to aluminum hydroxide gel. (ADP a8-26)

J. Attenuation of Representative Types of Foot-and-Mouth Disease Virus

Concentrations of glycidaldehyde (GDA) as low as 0.005%, inactivated high titered FMDV of guinea pig vesicular fluid origin. A 0.05% concentration was effective in less than 30 minutes. No GDA-resistant virus particles were detected during the inactivation studies. Inactivation rate was directly related to the ambient temperature up to 42°C and virtually disappeared at 5°C.

Glycidaldehyde compared very favorably with acetyleneimine as an inactivant for FMDV.

The neutralization of GDA by sodium thiosulfate is not instantaneous and concentrations as high as 10% failed to neutralize the virucidal activity of GDA.

The reactivity of GDA-treated virus with specific immune serum was essentially the same as for control preparations. CF titers as high as 1:240 were consistent in both GDA-treated and untreated antigen.

The precipitin pattern, using the agar-gel technique, did not indicate virus breakdown. Precipitin antibody was detected 7 days postinoculation from a single injection of GDA-inactivated virus. (ADP a8-27)

K. Survival and Inactivation of Foot-and-Mouth Disease Virus in Meat and Meat By-Products

A total of 42 Argentine cattle, repeatedly vaccinated against FMD with tri-valent vaccines (Types A, O and C), were selected for this experiment and tested in 3 groups. Each group of 14 vaccinated and 5 unvaccinated (control) mature cattle was infected with one of the 3 types (either A, O or C) FMDV. They were slaughtered 30 to 34 hours after infection. One lymph node was removed from one hind leg of each steer at slaughter. The corresponding lymph node from the other leg was obtained after ripening (chilling) the whole carcasses for 3 days. Several other lymph nodes were packed between meat chunks in wet salt-cure in barrels.

All lymph node samples and barrels of cured meat were shipped under strict safety precautions to the Plum Island Animal Disease Laboratory, U.S.A., and tested for the presence of virus using the most sensitive methods known. FMDV was detected in all fresh and all but one ripened lymph node sample from 15 unvaccinated (control) cattle. Virus also was found in 4 of 15 salt-cured samples held at 4°C, 38-39 days. After infection, 2 of 42 vaccinated Argentine cattle developed tongue lesions containing virulent virus. Foot-and mouth disease virus was isolated from a lymph node in 1 of these 42 unvaccinated cattle.

In view of these results, it was concluded that FMDV may be present in the lymphatic system of vaccinated, and subsequently infected cattle. Presently

available vaccination methods do not prevent the dissemination of FMDV through meat.

Using 7 known types of FMDV, A, O, C, SAT-1, SAT-2, SAT-3, and Asia 1, it was shown that bovine lymph nodes contain virus as early as 12 hours and as long as 15 days after inoculation. While considerable amounts of FMDV may be present in lymph nodes, it might be difficult to diagnose the disease by routine inspection procedures at the preclinical and convalescent stages of infection. Cattle slaughtered during the course of inapparent infection may propagate FMDV through animal products.

Foot-and-mouth disease virus was detected in joints of infected cattle and survived in synovial fluid of infected carcasses for 19 days when stored at 4C. Virus remained infectious for several weeks in joints stored successively at chilling, freezing, and thawing temperatures.

Foot-and-mouth disease virus was remarkably stable in blood and infected or contaminated animal tissues which had been spread on materials used to package meat (wood, paper, metal). In several tests, the virus survived 48 days in blood spread on a can and stored at 4C. These preliminary results indicated that meat shipping containers may play a significant role in disseminating FMDV. (ADP a8-28)

L. Biological Mechanism of Natural Resistance and Susceptibility to Foot-and-Mouth Disease Virus

Infant mice are highly susceptible to FMDV but develop a pronounced resistance as they mature. However, during the period of late pregnancy to about two weeks postpartum, 50-70% of female mice will succumb to FMDV. Factors which might be related to the mechanisms of these responses have been investigated:

1) Suspensions of minced kidneys from individual 1-week-old mice, which are uniformly susceptible to FMDV, produced virus quickly and to high titers with only slight variation between preparations. In cells from less susceptible 5-week-old mice, however, there was considerable variation between preparations in time when multiplication began, time of peak titer, and amount of virus produced.

2) Mother mice with litters reacted with much less sensitivity after passive transfer of serum from mice sensitized with bovine serum than did similar mothers following removal of litters or nonmated controls. Similarly, serum from sensitized mother mice with litters produced less sensitivity in nonmated female mice than did sera from mother mice without litters or from nonmated control mice.

3) After being subcultured eight times cells originating from calf kidneys were less susceptible to FMDV than cells from the primary culture. Experiments indicate that selection of resistant cells occurs during subcultivation and that many serial passages of FMDV in primary cells results in the selection of virus with more virulence for the subcultured cells.

(ADP a8-29)

M. Biological Alterations of Foot-and-Mouth Disease Virus from Continual Residence in Cell Cultures

Type C₃ Rio foot-and-mouth disease virus and several line of type A-119 have been established in chronic residence on cultured bovine kidney cells. In all instances, a reduction in virulence has been effected. Total virus in work harvests from calf kidney cultures ranges from $10^{6.5}$ to $10^{8.6}$ TCID₅₀. The amount of virulent virus in the various harvests (as determined by intralingual inoculations of cattle) ranges from 10^2 bovine ID₅₀ to 10^5 bovine ID₅₀ depending on time in chronic residence and degree of modification. Indications are that the loss in virulence results from virus selection rather than a mutation or genetic change.

Conventional rapid serial passage of one line of modified virus 14 times in calf kidney cultures resulted in an increase of one log of residual virulent virus in the final harvest ($10^{2.0}$ to $10^{3.1}$ ID₅₀) and partial restoration of the plaque forming ability ($10^{5.7}$ as compared with $10^{7.5}$ TCID₅₀). However the plaques were very small compared with average plaque produced by normal virus.

Virus populations with reduced virulence for cattle also had reduced virulence, but significant antigenicity for sheep.

Resistant cell lines developed from cultures cured of chronic infection may have value for use in more rapid selection of avirulent virus populations.

(ADP a8-30)

N. Morphological Aspects of Virus-Cell Relationships

Work was begun on the initial phase of the project, namely, development of primary or permanent cell lines which would react slowly to FMDV, or replicate the virus without destruction of the cells. Cultures of this type would be advantageous to morphological studies of virus-cell relationships.

Four primary cell lines were developed: two of bovine origin, one of swine origin and one of canine origin. All have low susceptibility to FMDV except the one of canine origin which appears to be refractory. Two of the lines were developed after suppression of the highly susceptible cells in the original cultures by viral action (in connection with ADP a8-30). None of the cultures have been carried long enough to determine if they have the indefinite growth potential of permanent cell lines. The cultures are being

observed for degree of susceptibility, conditions related to spontaneous cure of infection, and susceptibility to reinfection after cure.

(ADP a8-31)

O. Investigations of Rinderpest in Cattle

Rinderpest virus, strain, Kabete O, was attenuated in tissue cultures. An avirulent virus population was segregated using the terminal dilution technique. The virus has been studied in laboratory tests and has shown promise as a vaccine in cattle and sheep. It has not shown properties to revert to full virulence in either species. The thermal and hydrogen-ion properties of rinderpest virus have been determined using cell culture techniques.

A sample of the rinderpest vaccine developed at PIADL has been made available to a Food and Agriculture Organization Laboratory of the United Nations located in Cairo, Egypt. The vaccine is being studied in that country for its usefulness in protecting animals against exposure to rinderpest.

(ADP a8-23)

P. Studies on Foot-and-Mouth Disease Virus (PL 480 Project)

Research is being conducted on FMDV at the Instituto Biologica, Sao Paulo, Brazil. At this Institute the investigators are serially passaging certain types of FMDV in a variety of types of tissue cultures. Following passage, the viruses are examined to determine the immunizing properties and according to the results recently received from that Institute, there is evidence that at least some of the viruses are becoming somewhat attenuated for cattle. The workers at this Institute have shown that tissue cultures are exceptionally valuable in diagnosis of samples submitted from the field. Using tissue cultures as an indicator medium and saliva from affected animals as a test sample, positive identification of FMDV has resulted in a large percentage of the samples so examined. The workers at this Institute have also developed a cell line from the kidneys of swine which is useful in primary isolation of virus from samples submitted from the field. The cell line which has been developed by workers at this Institute multiplies rapidly, may be easily subcultured, and is sensitive to all of the types of FMDV against which it has been tested. It may well be useful for production of virus for commercial fabrication of FMD vaccine.

(S3-ADP-2)

Q. Studies on Various Indigenous Types of Foot-and-Mouth Disease Virus, and the Production of a Vaccine for the Control of FMD in Turkey (PL480 Proj)

This investigation is in progress under a PL 480 Grant to The Ministry of Agriculture, Laboratories of Foot-and-Mouth Disease and Tissue Culture, Etlik, Turkey. The work is still in the preliminary stage, since the grant was made during the reporting period.

(A22-ADP-8)

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PARASITES AND PARASITIC DISEASES OF CATTLE
Animal Disease and Parasite Research Division, ARS

Problem. The cost of parasitic diseases to the cattle industry of the United States is estimated to be in excess of \$400 million annually. Disorders caused by parasites are ubiquitous, generally insidious and often overlooked entirely. Diagnosis is difficult and successful treatments for many of these diseases are not available. Moreover, management practices to avoid spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling or eradicating parasitic diseases so as to provide for healthy cattle, insure adequate supplies of parasite-free beef for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a more prosperous agriculture and the national economy.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving biochemists, microbiologists, parasitologists, pathologists and veterinarians engaged in both basic and applied studies directed to the development of measures for the solution to the high and extremely costly incidence of parasitism in cattle. Research is being conducted on parasitic diseases at the following designated locations.

The Federal scientific effort devoted to research in this area totals 21.5 professional man-years. This effort is divided among subheadings as follows:

Ecological Factors Influencing Gastro-Intestinal Nematodes of Cattle 1.0 at the Animal Disease and Parasite Research Division, Regional Animal Disease Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Effect of Pasture Mixtures and Pasture Management on Control of Internal Parasites 1.5 at the Regional Animal Disease Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Acquisition and Effects of Roundworm Parasites of Cattle as Influenced by Diet 1.5 at the Animal Disease and Parasite Research Division, Beltsville Parasitological Laboratory, Beltsville, Maryland.

Cultural Characteristics and Artificial Propagation of Protozoan Parasites 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Host-Parasite Relationship of Coccidial Parasites of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Ecology and Immunology of the Cattle Lungworm, Dictyocaulus viviparus 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Clinical and Physiological Aspects of Roundworm Parasitism in Cattle, Including Anthelmintic Treatment 2.0 at the University of California, Davis, under a cooperative agreement with the ARS-USDA.

Investigations of Trichomonad Parasites 1.0 at the Animal Disease and Parasite Research Division Regional Animal Disease Laboratory, Logan, Utah, and under a cooperative agreement with the Utah Agricultural Experiment Station, Logan, Utah.

Host-Parasite Relationship of Intestinal Worms, Cooperia spp. in Cattle 2.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Epizootiological and Ecological Investigations of the Internal Parasites of Grazing Cattle 1.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Etiology and Immune Response of Cattle to Winter Coccidiosis 1.0 at the Regional Animal Disease Laboratory, Logan, Utah, and under a cooperative agreement with the Montana Agricultural Experiment Station, Bozeman.

Anaplasmosis of Cattle 4.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland, and through a memorandum of understanding and other agreements in cooperation with the State Experiment Stations in California, Illinois, Louisiana, Nevada, and State Veterinarian of Tennessee, the USDA Entomology Research Station, Kerrville, Texas, and the Delta Branch Experiment Station, Stoneville, Mississippi.

Interrelationships of Diet and Parasitic Infection in the Production of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Histochemistry of Gastro-Intestinal Nematodes of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Parasites of Cattle with emphasis on Stephanofilarial Species 1.0 at the Animal Disease and Parasite Research Division Regional Animal Disease Laboratory, University Park, New Mexico.

Environmental Factors Influencing Parasites and Parasitic Diseases of Economical Importance in Ruminants (Cattle, Sheep, and Alpacas)(PL-480 Peru)

Investigations on Anaplasmosis, Piroplasmosis and Babesiallosis of Cattle are under way through a PL 480 Grant at the School of Veterinary, Montevideo, Uruguay (PL 480 Uruguay)

PROGRAM OF STATE EXPERIMENT STATIONS

Twelve Western States and the Department are cooperating in regional research on internal parasitological problems of cattle (W-35). Informal coordination is maintained with States in the southern region also working on this subject. New and improved methods for diagnosing nematode parasitic diseases are being developed and relationships between types of pasture forages and degree of parasitism are being established. Biological and chemical controls are under evaluation. The effects which promising anthelmintics have upon weight gains and feed efficiency of parasitized cattle are being measured.

Basic studies are seeking to establish how nematodes damage the host animal, interfere with nutrition and bring about disease. Studies of biochemical systems involved in parasite metabolism and the effect of anthelmintics on these systems are providing key information necessary in developing improved therapeutic measures.

Other studies are aimed at reducing exposure to cattle parasites through the development of systems for managing grazing and feeding procedures. Factors which favor over-winter survival of infective parasite larvae are being determined and micro-climatic conditions conducive to larval infectivity are being established. Studies at several locations are in progress on coccidiosis of cattle to determine the conditions favoring outbreaks of this disease. Factors affecting immunity to this parasite are being determined. Basic information is being sought at a number of States on the nature of Anaplasma in order to elucidate the life cycle of this parasite and provide a means for its control. Preventive immunization is under study and methods of eradication are being explored.

The total State scientific effort devoted to this research is 8.9 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Etiological Factors Influencing Gastro-Intestinal Nematodes of Cattle.

1. Investigations made at Experiment, Georgia, under the auspices of the Regional Animal Disease Laboratory at Auburn, Alabama, showed a reduction in the number of infected larvae of various cattle and sheep nematodes proportional to the increase in number of viable spores of Bacillus thuringiensis var. thuringiensis Berliner (Rohm and Hass Co., and Stauffer Chemical Co.,) incorporated in feces-worm egg cultures. Some of the same materials fed to calves and sheep proved to be toxic, without any appreciable reduction in the number of larvae obtained from fecal cultures from these hosts. (ADP bl-6(Rev.)

2. Studies have shown that a grain ration (corn) appears to have an inhibitory action on the development in the feces of the larvae of the five species of cattle nematodes (Trichostrongylus axei, T. colubriformis, Oesophagostomum radiatum, Cooperia oncophora, and C. pectinata.) Apparently, silage does not have any effect on larval development. (ADP bl-6(Rev.)

3. Sporangia of a mold, Pilobolus spp. was observed to disseminate infective larvae of nematode parasites of cattle present on the vesicle at the time the sporangia were discharged. This may constitute another way for the dissemination of nematode larvae from the fecal pad in the field to the forage to be consumed by ruminants. This report confirms earlier observations made in England. (Alabama and Georgia) (ADP bl-6(Rev.)

B. Acquisition and Effects of Roundworm Parasites of Cattle as Influenced by Diet.

At the Beltsville Parasitological Laboratory (BPL), Beltsville, Maryland, four experiments, using a total of 37 calves, were performed to determine whether feeding a normal level or a subsistence level of combinations of grain and hay for 7 weeks preinoculation of larvae would effect their resistance to infection of gastro-intestinal worms. The calves weighed from 171 to 331 lbs. at the time of inoculation. Lots 1 and 2 received 248,000 and 116,000 larvae, respectively, of the medium stomach worm. Lots 3 and 4 received a total mixture of 485,000 and 523,000 larvae of the medium stomach worm and 6 other species of gastro-intestinal parasites, respectively. Calves were necropsied 6 to 7 weeks post-inoculation.

The parasitized calves on the low feeding level were outgained by their respective controls by 12.2 lbs./100 lbs. of TDN consumed (total digestible nutrients) (Avg. of all 4 experiments). The parasitized calves on the higher feeding level were outgained by their controls by 9.2 lbs./100 lbs. of TDN consumed. The parasitized calves on the higher feeding level outgained those on the lower feeding level by 11.8 lbs./100 lbs. TDN consumed and the uninfected control calves on the higher feeding level outgained those on the lower feeding level by 11.5 lbs./100 lbs. TDN consumed.

Analysis of these results showed that moderate infections with gastro-intestinal nematode parasites reduced the efficiency of feed utilization by calves more than did mild infections, but feeding level did not appear to affect the susceptibility of the calves to infection with these parasites under the conditions of the experiments. (Maryland) (ADP bl-19 R)

C. Cultural Characteristics and Artificial Propagation of Protozoan Parasites.

Experiments at the Beltsville Parasitological Laboratory on the symbiotic relationship between the protozoan parasite, Histomonas meleagridis, and the enteric (intestinal) bacterial flora of its natural hosts, chickens and turkeys, and from several small mammals, showed bacteria from the turkey were more beneficial to the parasite than were those from the chicken; those from small mammals were without benefit. This finding may provide at least a partial explanation of why this parasite is more damaging to the turkey than to the chicken.

The probiotic activity for Histomonas of turkey cecal bacteria was not destroyed by heating at a temperature of 56°C (132.8°F) for 30 minutes or at lower temperatures for as long as 60 minutes. At temperatures of 65°C (149°F) or higher the activity of the bacteria was destroyed within about 5 minutes. (Maryland) (ADP bl-22)

D. Host-Parasite Relationships of Coccidial Parasites of Cattle.

The ADP Regional Animal Disease Laboratory, Auburn, Alabama, reported that for the first time observations of a preliminary nature were made on the endogenous cycles of Eimeria cylindrica and E. canadensis in calves. Schizonts, merozoites, microgametocytes and macrogametocytes of E. cylindrica were found in fresh smears and sections of the intestines at 8 days postinoculation. In E. canadensis at 15 days postinoculation, fresh smears revealed microgametocytes and macrogametocytes in the small intestine 6 and 12 feet anterior to the cecum. (Alabama) (ADP bl-23(Rev.))

E. Ecology and Immunology of the Cattle Lungworm, Dictyocaulus viviparus.

Work conducted at the Beltsville Parasitological Laboratory showed that oral vaccination of calves with infective larvae of the equine lungworm for protection against infection with the cattle lungworm was without adverse clinical effect. The procedure showed promise of efficacy. (ADP bl-24)

F. Clinical and Physiological Aspects of Roundworm Parasitism in Cattle, Including Anthelmintic Treatment.

The School of Veterinary Medicine, University of California, Davis, under a cooperative agreement with the USDA, reported research on anthelmintic treatment with the following results:

I. ANTHELMINTIC STUDIES

- A. Trial 1. Activity of Bayer S-940 and Bayer S-6658 against nematodes in sheep.
 - 1. Bayer S-940 removed 95% and 98% of nematodes from lambs at dosages of 50 mg./kg. and 100 mg./kg., respectively.
 - 2. Bayer S-6658 removed 92% and 98% of nematodes from lambs at dosages of 200 mg./kg. and 400 mg./kg., respectively.
- B. Trial 11. Activity of Bayer 9017 and Bayer 9018 against nematodes in sheep.
 - 1. Bayer 9017 removed 65% and 90% of nematodes from lambs at 15 mg./kg., and 30 mg./kg., respectively.
 - 2. Bayer 9018 removed 17% and 38% of nematodes from lambs at 20 mg./kg. and 40 mg./kg., respectively.

II. PHYSIOLOGICAL STUDIES

- A. Iron kinetic studies in Hereford steers during the early acute phase showed no significant alterations from the normal. This would suggest that only in the later stages of the disease are alterations to be found.
- B. The biological half life of total body water in cattle suffering from acute gastrointestinal parasitism was found to be about twice that of normal animals.
- C. The serum albumin/serum globulin ratio was found to change from 0.49 in severely parasitized cattle to 1.00 twenty-five days after therapy. (California) (ADP 61-25)

G. Investigations on Trichomonad Parasites.

Research workers at the ADP Regional Animal Disease Laboratory, Logan, Utah, reported that a graded series of freeze-dried organisms was inoculated intravenously into two rabbits. Both rabbits showed anaphylactoid shock after each inoculation. One rabbit died after the third inoculation, and as a result, the other rabbit was given only four inoculations. The rabbit that died had a serum agglutination titer of 160 at time of death; the other rabbit produced an excellent serum titer of 2560. Serum from this rabbit, however, with the homologous antigen produced no precipitin lines on gel diffusion plates. Serum from the rabbit that died produced one precipitin line. Due to the high agglutination titer produced in the one rabbit, it is felt that further trials are warranted using freeze-dried T. foetus organisms intravenously.

Five experiments were run using different strains of Trichomonad foetus. A series of six inoculations of live washed Trichomonads, followed by a single large inoculation one month later, produced antisera with agglutination titers up to 2560 and 10240 in the first two experiments. The second inoculation did not increase the agglutination titer but did result in stronger precipitin reactions in gel diffusion plates. However, only two and three precipitin lines were formed. The antisera were freeze-dried and rehydrated to one-fourth their original volume. Gel diffusion reactions were considerably improved, producing 4 to 6 precipitin lines consistently. This concentrated serum also responded normally to electrophoresis and can be used for microimmuno-electrophoresis.

At the Utah Agricultural Experiment Station, Logan, research was continued under a cooperative agreement with the USDA on trichomonads and related flagellates of the bovine digestive tract. In the examination of animals, infection was found as follows: a) in the cecum of 1, and in the rumen of 1 of 45 cattle; b) in the cecum of 16 of 21 calves; c) in the cecum of 7 and in the rumen of 8 of 17 sheep; d) in the cecum of 14 of 14 pigs. Twelve pigs had Trichomonas suis; 7 had T. buttrey; and 4 had Trichomitus rotunda.

A pentatrichomonad was found to have the highest incidence of any flagellate in the bovine cecum and rumen. It was easily cultivated in several different media. Two strains from the rumen had similar growth curves, but in 2 clones from the cecum and 2 strains of Pentatrichomonas hominis of human and canine origin, the growth curves were distinctly different. An organism with 4 flagella arising in pairs was found in the cecum and feces of cattle, and described as Monocercomonoides bovis n. sp. (Utah)
(ADP bl-26)

H. Host-Parasite Relationship of Intestinal Worms, Cooperia species, in Cattle.

Reported research from the ADP Regional Laboratory, Auburn, Alabama, showed that calves and lambs inoculated with infective larvae of either of the intestinal nematodes, Cooperia oncophora or C. pectinata, developed immunity to challenge inoculation with either the homologous or heterologous species. One animal that failed to develop immunity to the homologous species also lacked immunity to the heterologous species.

Calves inoculated with 300,000 Cooperia pectinata infective larvae in a single dose were killed by their infections. Two that were moribund 6 weeks after inoculation had lost 20 and 21 pounds. Calves inoculated with 10 successive daily doses of 30,000 larvae each gained an average of 7.3 pounds, while non-inoculated controls averaged a 36.7 pound gain. Calves given 42 successive daily doses of 7,140 larvae gained an average of 31.5 pounds, while controls averaged 42 pounds in one test, and 77 and 78.3 pounds, respectively, in another similar test. The results are additional evidence of the pathogenicity of C. pectinata and indicate that clinical

parasitism develops in susceptible calves as the result of acquisition of infective larvae in large numbers over short periods of time. (Auburn, Ala.)
(ADP bl-27)

I. Epizootiological and Ecological Investigations of the Internal Parasites of Grazing Cattle.

The Beltsville Parasitological Laboratory research workers reported that the larvae of the gastrointestinal worms of cattle can develop at a temperature of 35°F to the second stage when they have been preconditioned for two weeks at 45°F. This is a much lower temperature than is usually thought to be conducive to the development of the gastrointestinal nematodes of cattle.

The viability of eggs of the beef tapeworm of man was reduced by 80 and 90% by exposure to 50,000 r and 100,000 r of x-irradiation, respectively. Only 0.29% of the cysts (Cysticercus bovis), which cause condemnation and retention of carcasses for bovine cysticercosis under meat inspection regulations, developed from a large dose of eggs exposed to 200,000 r of x-irradiation.
(Maryland) (ADP bl-28)

J. Etiology and Immune Response of Cattle to Winter Coccidiosis.

Research was continued at the Montana Veterinary Research Laboratory, Agricultural Experiment Station, Bozeman, under a cooperative agreement with the USDA. Reported observations on 10 disease outbreaks in cattle indicated that Eimeria zurnii was the predominant organism in 5 cases, E. bovis in three, and E. canadensis and E. brasiliensis each in one case. Confirmatory diagnosis of clinical coccidiosis was made in only 4 instances in which E. zurnii occurred alone. Oocyst counts on mucosal scrapings from the lower colon of the above animals varied from 19,500 to 3,612,000 oocysts/gram.

Supernatant from a saline emulsion of colonic contents of a Hereford calf that died after showing symptoms typical of convulsive coccidiosis, was highly toxic to mice when injected intraperitoneally. This supernatant was not neutralized by Clostridium perfringens anti-sera of types A, C, or D, or by Cl. septicum antiserum. This colonic supernatant remained lethal to mice, after being stored for 6 days, when injected intraperitoneally in 0.1 ml. doses.
(Montana) (ADP bl-29)

Studies were continued on Winter Coccidiosis at the ADP Regional Laboratory at Logan, Utah. Three experiments were conducted involving prolonged daily oral inoculation of Holstein-Friesian calves with sporulated oocysts of Eimeria bovis and E. zurnii. In one experiment calves were inoculated 50 days with 500 or 15,000 E. bovis oocysts by adding an aqueous suspension containing oocysts to the evening feeding of milk. Each calf was given a single challenge inoculation of 500,000 oocysts after recovery from the initial prolonged inoculations.

In a second experiment, similar in objectives, calves were fed 100 or 15,000 oocysts daily in the evening feeding of milk, or 1000 oocysts in the evening feeding of grain. A third group of calves served as controls. All calves were given a challenge inoculation of 500,000 oocysts.

The results of the first 2 experiments showed that calves ingesting the least oocysts developed less severe symptoms of coccidiosis than did those ingesting the larger number. The length of time calves were susceptible to repeated inoculations was about the same in all groups and the degree of immunity was similar, although calves undergoing infections wherein large numbers of oocysts were discharged and clinical signs were severe seemed to exhibit a somewhat stronger immunity. These results confirm those reported from one experiment in last year's report.

Ten calves were inoculated with sporulated oocysts previously exposed to radiation of 10,000 r, 50,000 r, 100,000 r, or 200,000 r in a cobalt-60 source. Calves receiving oocysts irradiated at 10,000 r developed coccidiosis similar to control calves receiving non-irradiated oocysts. Those receiving oocysts exposed to 50,000 r exhibited mild coccidiosis. Those receiving oocysts exposed to 100,000 r or 200,000 r developed no evidence of coccidiosis and were completely susceptible to challenge with non-irradiated oocysts. It appeared that the sporulated oocysts exposed to 100,000 r and 200,000 r were killed by the radiation and were unable to elicit an immune response in the gut. The amount of radiation required to kill sporulated oocysts appears to be between 50,000 r and 100,000 r, probably about 75,000 r. (Utah) (ADP b1-29)

K. Anaplasmosis of Cattle

At the Beltsville Parasitology Laboratory, research workers reported the following findings: Serum samples from cattle in the incubative, acute, and carrier stages of bovine anaplasmosis were tested by the agar gel precipitin technique and the complement-fixation reaction. The agar gel technique proved to be unreliable as a supplementary diagnostic test.

A free soluble antigen of Anaplasma marginale, exo-antigen, was found to be produced and released into the peripheral blood of cattle with acute anaplasmosis. The significance or immunogenic potential of this material has not been determined.

Calves given an immunizing inoculation of sonicated hemolysate of anaplasma-infected RBC (red blood cell) in a mineral oil adjuvant and then challenged were only partially protected. The hemolysate conferred partial protection against the severe form of the disease but did not prevent the animals from becoming carriers.

Filtration and high speed centrifugation with a sucrose gradient were employed in the examination of the complement-fixation antigen to determine whether sub-microscopic particles of Anaplasma occur in this antigen. Sub-microscopic units of A. marginale were not found.

Ten adult-to-larva and two series of adult-to-adult hereditary transmission experiments with the Rocky Mountain wood tick, Dermacentor andersoni Stiles, failed to transmit anaplasmosis. All test calves were found to be susceptible when challenged with anaplasma-infected blood.

Exposure of adult ticks to hibernating environments of 4°C, relative humidity of 40-50%, and of 25°C, relative humidity of 80%, had no observable effect on their transmission potential. However, ticks exposed to the low temperature fed faster and produced 50 - 70% more eggs after hibernation than did ticks held at 25°C. Nine of 49 D. andersoni males, subjected to hibernation for 263 days, survived. Of these, 5 attached and fed on a susceptible calf which failed to contract anaplasmosis. The ticks had originally fed on a calf with acute anaplasmosis 358 days before they were placed on the test (susceptible) calf.

Colonization of D. occidentalis has been successful and the colony is now in the F₃ generation. (Maryland)

The ARS anaplasmosis research herd at Kerrville, Texas, is composed at the present time of 40 mature cows, 8 two-year-old heifers, and 27 calves nearing weaning. The last of the reactor cattle were sold last year (1963) and the herd has continued through this fiscal year as an anaplasmosis-free herd. The total number of cattle will be reduced to approximately 35 to conform to available pasture. (Texas) (ADP bl-30)

L. Interrelationship of Diet and Parasitic Infection in the Production of Cattle.

Research workers at the ADP Regional Laboratory, Auburn, Alabama, reported the effect of parasitosis on the basal metabolic rate of rabbits infected with either 5,000, 10,000, 15,000, 20,000, or 25,000 infective Obeliscoides cuniculi larvae, indicated that the parasitic infections established did not cause a marked difference between the basal metabolism of the infected and control animals.

Experiments on the biology and host-parasite relationship of Longistate noviberiae and Trichostrongylus affinis in domestic rabbits indicate these parasites are well adapted for experimental use. The short prepatent period, direct life cycle, high percentage of adult worm recovery makes these ideal parasites for experimental work with diets in rabbits. (Auburn, Alabama) (ADP bl-31)

M. Histochemistry of Gastro-Intestinal Nematodes of Cattle.

The report on work conducted at the ADP Regional Animal Disease Laboratory, Auburn, Alabama, showed that 8 to 10 days after infection of cattle with the nodular worm, Oesophagostomum radiatum, there is a decrease in collagen around the lesions formed by the worm in the walls of the ilium. Concurrently with this decrease in collagen there is a real or apparent increase in a substance, probably glycoprotein, containing protein and carbohydrate moieties, between the lesions and the lumen of the intestine. Such histochemical changes were not observed around the sites of infection by the medium stomach worm, Ostertagia ostertagi, 8 days after the host calves were infected. At the above stages of these diseases, alterations in the distribution of glycogen and acidic mucopolysaccharides of the tissue were not observed.

Glycogen and acid mucopolysaccharides, as well as collagen, have been found in Obeliscoides cuniculi, a nematode of the domestic rabbit. Glycogen was principally found in the intestinal wall and in the body wall muscles. Acid mucopolysaccharides lined the digestive tract and were present in the muscles of the body wall. Collagen was seen in the cuticle, the hypodermis, and in various membranes, as well as in the gonads. (Alabama) (ADP bl-32)

N. Parasites of Cattle with Emphasis on Stephanofilarial Species.

Investigations made at the ADP Regional Animal Laboratory at University Park, New Mexico, were reported as follows: Stephanofilaria stilesi is a small filarioid nematode causing an ulcerative dermatitis along the ventral mid-line of cattle, and uses the horn fly, Haematobia irritans, as a biological vector. Horn flies were experimentally infected with the larval stages of S. stilesi by exposing laboratory-reared flies to the lesion on infected cattle. The infective stage is reached after about 18 days of development in the fly. The biological cycle of S. stilesi was completed by exposing young calves to infected horn flies. Two calves developed lesions typical of stephanofilariasis within two weeks after their initial exposure. One calf was examined post-mortem after one month and found to be infected with immature S. stilesi. From eight to thirty-two per cent of the horn flies collected from infected cattle on rangeland, irrigated pasture, and in drylot were found to be infected with the larvae of S. stilesi. (New Mexico) (ADP bl-33)

O. Under a PL 480 Grant to the School of Veterinary Medicine, University of San Marcos, Lima, Peru, research is in progress on Environmental Factors Influencing Parasites and Parasitic Diseases of Economical Importance in Ruminants (Cattle-Sheep-Alpacas). Most of the work reported has been of the nature of a preliminary survey of multiple areas or districts to determine the kinds of parasites therein that infect animals.

P. Under a PL 480 Grant to the School of Veterinary, Montevideo, Uruguay, research is in progress on Anaplasmosis, Piroplasmosis, and Babesiellosis of Cattle. Crushing and macerating of tick larvae appeared necessary for development and evolution of the larvae, and their transformation into nymphs in vitro. A temperature of 37°C was found to be the most favorable for cell survival and development.

The development of tissular components was improved by the addition of glutamine to the media. Chicken plasma was also found to improve the media.

The pathogenicity of whole blood infected with Babesia bigemina was modified by irradiation with gamma rays at dosages above 30,000 r.

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DAIRY CATTLE INSECTS
Entomology Research Division, ARS

Problem. Flies, mosquitoes, grubs, lice, and ticks are common pests of dairy cattle that cause important losses in all parts of the United States. Heavy attacks by biting flies lower milk production by 5 to 20%. Total losses to dairy cattle attributable to insects and ticks are estimated to exceed \$200 million annually. Certain insect pests are also involved in the transmission of diseases of dairy cattle. Methods of control for dairy insects have received setbacks during recent years because the best available insecticides and most promising new materials produce residues in milk. In addition, house flies around dairy establishments have developed resistance to DDT and other insecticides. There is, therefore, great need to find safe, effective, non-residue insecticides and repellents to control these insects and ticks. Effective systemic insecticides and ways of administration which would avoid residues are needed to combat grubs in dairy cattle and to prevent the face fly and horn fly from breeding in the manure. New approaches to control, including radiation and chemosterilants, need to be explored further to determine their feasibility for the control of several dairy-cattle pests. Research should be continued to support the Southwestern screw-worm eradication campaign. Efforts also should be made to find and evaluate insect pathogens, parasites, and predators for controlling certain dairy-cattle pests. Expanded basic studies on the biology and physiology of these pests are needed to find weak links in their life cycle to serve as a basis for the development of more effective and safer methods of control. Research is also urgently needed on the role of insects in the spread of diseases of dairy cattle.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program involving basic and applied research on insects and ticks which affect the health and productivity of dairy cattle. Studies are conducted on the biology, physiology, genetics and nutrition of the screw-worm fly, stable fly, horn fly, horse and deer flies, the face fly, mosquitoes, and other pests; on the nature of insect resistance to insecticides; on the mode of action of insecticides and on their absorption, metabolism and excretion by insects; the effects of irradiation and chemosterilants on insects; insect attractants and repellents; and other new approaches to control. Research is directed towards the development of more effective conventional and systemic insecticides and protective treatments for the control of dairy-cattle pests. Studies are conducted to determine the occurrence of insecticide residues in the tissues and the milk of treated animals. Minor attention is given to the development of sanitation and management procedures and to biological control, especially parasites and predators, for controlling the face fly, stable fly, horse fly, and several other pests. Studies are conducted in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions to develop physical and mechanical methods of control, to evaluate traps and devices for estimating and

controlling natural insect populations and improved or special equipment for the application of insecticides to dairy cattle. Limited research is conducted on the role of insects and ticks as vectors of animal diseases, with special emphasis on bovine anaplasmosis. The research is conducted in major laboratories at Kerrville, Tex., Corvallis, Oreg., and Gainesville, Fla., and at satellite stations at Beltsville, Md., Stoneville, Miss., Lincoln, Nebr., and Fresno, Calif.

The Federal scientific effort devoted to research in this area totals 16.6 professional man-years. Of this number 6.4 is devoted to basic biology, physiology and nutrition; 3.7 to insecticidal and sanitation control; 2.5 to insecticide residue determinations; 0.3 to biological control; 1.9 to insect sterility, attractants and other new approaches to control; 0.3 to evaluation of equipment for insect detection and control; 0.7 to insect vectors of diseases; and 0.8 to program leadership.

PROGRAM OF STATE EXPERIMENT STATIONS

Valuable information on insects affecting dairy cattle is being provided by research in the States. Studies are in progress to determine the abundance, geographical distribution, seasonal variations and economic importance of pest species. Rearing methods are being developed to provide insect specimens (1) for laboratory studies involving the effects of ecological factors on growth and survival; (2) for studying the micro-organisms normally present in pest insects; (3) for cattle disease transmission tests performed to determine which insects may serve as vectors; and (4) for control studies.

Various substances are being evaluated for their attractant or repellent effects on such pest insects as flies. Those attractants which exert a significant effect are incorporated as baits with new insecticides or chemo-sterilants. Various other application methods are also being evaluated.

The development of resistance to insecticides in flies has brought about research to determine methods of combatting it as well as initiating a search for new chemicals. Studies are in progress to determine the effects of repeated heavy insecticide dosages as opposed to light doses, and the influence of fly behavior, development and reproductive capacity on resistance. The mechanism of resistance in the insect in relation to penetration of the integument, distribution, activation, degradation and excretion of the insecticides is also being investigated.

Biological control research is being performed to determine the value of natural agents as supplementary control measures. On dairy cattle, materials and techniques of application are being tested for their effects on weight gain, milk production, milk contamination and animal health as well as pest control. Milk and tissues are being recovered from treated animals and examined for pesticide content. Detection of metabolites as well as the original compounds is being emphasized.

There are 6.7 man-years devoted by the States to research on insects affecting dairy cattle.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Basic Biology, Physiology and Nutrition

1. Mosquitoes. At Gainesville, Fla., studies on mosquitoes have been continued to elucidate basic biology fundamental to development of new and effective control techniques. Extensive laboratory and field research has shown that males of a laboratory colony of Anopheles quadrimaculatus do not disperse as readily as do males of the wild type. Laboratory crosses accomplished through forced copulation of the laboratory and wild strains have developed a hybrid strain which mated readily in the laboratory in the F₃ generation. Field studies on the wild strain of quadrimaculatus in Central Florida showed this species to consistently and predominantly consist of young females (females in the process of laying their first batch of eggs). The only factor that influenced the size of the population was the availability of larval breeding sites. Eggs of this species of mosquito could be stored at 58°F. and 100% R.H. for seven days without decreasing their viability. Preliminary attempts to establish laboratory colonies of Aedes infirmatus, Psorophora ferox, and Culex nigripalpus were unsuccessful.

At Corvallis, Oreg. studies were continued on the biology and ecology of mosquitoes. The occurrence and distribution of Aedes increpitus in the Willamette Valley was followed by sampling areas in Suver community considered representative of larval breeding sites in the Willamette River from Eugene to Portland. All larval instars were found at weekly intervals from January through March 1964.

Laboratory studies in Oregon showed survival of Aedes sierrensis larvae for at least one year. Thirty-one individuals survived when maintained at temperatures of 41° to 46°F. When the temperature approached 46°F a few of the larvae pupated and adults emerged. At the end of the year all individuals pupated or emerged from pupae when removed to room temperatures.

Studies on mosquito biology were continued in California. Continuous rearing of Uranotaenia anhydor in the laboratory was accomplished. Eggs hatched in 2.5 to 3.5 days at 75°F, larval and pupal stages required 2-3 days each with little mortality noted at 77°F. The maximum life span of a male and female was 49 and 55 days, respectively. Mating occurred readily and the preovipositional period was 4-5 days at 70°F.

Studies have led to a much better understanding of the ecology of Orthopodomyia californica. Contrary to suggestions in the literature, there appears to be little opportunity for competition between O. californica and Aedes sierrensis because of their different ecological preferences. O. californica is not a rare species. Larval stages were collected every month of the year. Overwintering occurs as 2nd - 4th instar larvae. O. californica

was associated with constant or decreasing water levels in tree holes of cottonwood and willow which are phreatophytes. Aedes sierrensis require fluctuating water levels for egg hatch. Evaluations of adults from field-collected larvae of 9 species of mosquitoes revealed autogenous egg deposition by only one species - Aedes pullatus.

Studies have confirmed that the major factor responsible for DDT-resistance in the mosquito, Culex tarsalis, is the increased ability to detoxify DDT by oxidative metabolism.

2. Stable flies. In Texas studies were conducted in an effort to improve procedures for rearing stable flies and to determine the effects of adverse environment on larval and pupal development. A medium consisting of 30 parts dry C.S.M.A. medium and 100 parts of water (wt/wt) was about optimum for larval survival and development. Lesser and greater amounts of water adversely affected larval survival and development. Second stage larvae survived under a wider range of moisture content than first stage larvae.

In Texas studies were conducted to determine the relative numbers of eggs that mated and unmated female flies would lay by placing individual virgin flies in cages and pairs of males and females in cages. Mated females began laying on the 6th day and laid an average of 112.6 eggs, whereas only 2 of 10 unmated females oviposited beginning on the 17th day, and the average number of eggs was only 5.4. The average life of mated and unmated flies was 25.9 and 19.5 days, respectively. One-day old male stable flies do not mate. The mating percentages for 2- and 3-day old males was 40 and 75, respectively.

At Beltsville, Md., the mating behavior and reproductive capacity of stable flies were studied. Successful matings of flies occurred between one-day-old males and five-day-old females and between one-day-old females and five-day-old males. The females begin laying eggs when eight days old and lay up to 600 during a lifetime which may be as long as 4-5 weeks.

3. Face fly. In Nebraska, various combinations of sugar, dried milk, whole milk, egg yolk, citrated blood, dry blood, pollen, and diamalt were tested in an effort to develop an improved diet for colonies of adult face flies. A semiliquid mixture of sugar (6 parts), dry milk (6 parts), egg yolk (1 part) and citrated blood proved most satisfactory, with females producing an average of 26 eggs. Egg production was slightly less when water was substituted for citrated blood.

In Nebraska laboratory studies showed that crowding of face fly larvae in rearing containers reduced the size of the pupae but did not affect the percent of adult emergence. When 100, 200, and 300 larvae were used, the average weights of the pupae were 29, 26, and 21 milligrams, respectively.

In studies of the effect of low temperature, mortality of face fly pupae stored at 40 and 45°F was light to moderate for 3 weeks but was almost

complete after 5 weeks. Storage at 36-38°F caused considerable mortality in 1 week and almost complete mortality in 3 weeks. There was very little difference in the survival of 1, 2, 3, and 4 day-old pupae.

Laboratory observations showed that both sexes of the face fly begin mating when about 48 hours old. Adults mated up to 5 times in one day and 10-15 times on successive days. Mating time ranged from 2 minutes to almost 5 hours, averaging 1.0-1.5 hours. Females with sperm in the spermathecae mated an average of 80 minutes, whereas those without sperm mated an average of 42 minutes.

In Nebraska adult face flies were first observed on farms on May 13, but pupae were not found until May 20. Thereafter, adult and pupal numbers increased steadily until late August, then declined rapidly except on one farm. Very few adults or pupae were present by mid-October. There were great differences in the adult and larval populations on different farms but the factors responsible were not determined. Parasitism of pupae averaged only 0.6%.

In Nebraska ecological studies of cattle droppings in pastures showed a total of 15 species of dipterous larvae. Face fly larval populations were small in May, predominant in June, July, and August, small during September and disappeared by mid-October. Sarcophagid larval breeding (4 species) followed the same trend as the face fly through August but populations were still high in October after face fly larvae disappeared. Over 30 species of Coleoptera were collected over the season, with Aphodius making up over 50% of the total population. Pupal parasitism (Hymenoptera) was relatively light and concentrated in Sarcophagids.

During late September and October, studies in Nebraska showed that released marked adult face flies migrated up to 2 miles within a few days. Marked flies were captured for about 2 weeks after being released. Adult face flies began hibernating on September 13 when the average daily temperature dropped to 56°F. Practically all flies had disappeared from the field by October 11 and an estimated 100,000 flies were observed hibernating on the walls and roof in one barn. Flies were not active even though the temperature was 78°F in the barn. Over 90% of flies collected from the barn in early December and stored at 36-38°F survived but only 4% of those stored under hay in the barn survived the winter. Mortality was at least 99% under normal hibernating conditions where minimum temperatures were as low as -15°F early in January.

Studies at Beltsville, Md., showed that the face fly can complete development from egg to adult in 10 to 12 days (egg stage -- 1 day, larval stage -- 4-5 days, pupal stage -- 5-7 days). Females lay fertile eggs within four days. Eggs are deposited in batches of approximately 20 at 2- to 8-day intervals. Females can lay fertile eggs throughout their life after only a single mating.

4. Horn Fly. In Texas, studies were conducted to compare the suitability of manure from cattle fed alfalfa, sorghum, and prairie hay for the development of horn fly larvae. More larvae matured in the alfalfa manure and the pupae were significantly larger than in the other manures. Sorghum manure was somewhat more satisfactory than prairie hay manure.

5. Screw-worm. Research on the screw-worm continued at Mission, Tex., in support of the sterile male release program. A line of traps along the Colorado and Concho Rivers was used to determine the flight range of sterile, laboratory reared screw-worm adults marked with P-32 or dyes. In the initial experiment in May and June 1963, over one million flies were released. The most flies were recaptured within 50 miles of the release point, but one was trapped at 65 miles, four at 80 miles, two at 110 miles, two at 140 miles, one at 165 miles, one at 170 miles, and one at 180 miles from the release point. These studies indicated that a barrier (sterile fly release zone) to keep fertile flies from reaching Texas would need to extend at least 200 miles into Mexico. Additional studies showed that flights in hot weather (June and July) were relatively short and that maximum dispersal occurred in spring and early summer, and again in September and October.

Research was continued to find and develop genetically distinct strains of screw-worm flies. In one study, about 229,000 adults of the Florida strain were examined for inheritable characters. Breeding and backcrossing studies with candidate mutants showed the presence of several strains. Tretamine (a known chemical mutagen) and irradiation were used to induce mutations. In these studies, 23 new strains were produced. Six strains showed unusual larval characters, involving the number of spiracles or the spines on the 11th abdominal segment. All the strains were studied for hardiness and competitiveness and 7 strains discarded when they showed no promise of ever becoming suitable for field use.

Through the SAG test, a technique reported previously for determining mating aggressiveness of sterilized or mutant screw-worm flies, it was determined that one strain of Texas screw-worms contained highly competitive males. Male flies produced in the plant operated by the Animal Disease Eradication Division on beef lung were as aggressive as males from the Entomology Research Division's research colony reared on the standard horse meat diet. Male flies reared in an experimental hydroponic medium were also equivalent in mating ability to flies reared on horse meat.

Studies were continued on the effects of desiccation, starvation, and age of flies at time of release on survival. In studies with a substrain of the original Florida strain of flies selected for resistance to starvation (food and water), adults of the 16th to 19th generations survived as well for 96 hours as the original strain survived for 56 hours. At about 50 hours of starvation, 50% of the unselected strain flies were dead, but only 0.3 to 1.7% of the 19th generation of the new substrain. Sexual aggressiveness of the males in the new substrain appeared normal until the 20th generation when a drop in aggressiveness occurred.

A possible taxonomic difference between laboratory-reared screw-worms and wild screw-worms has been found. Further studies are needed to determine whether this larval characteristic is statistically valid.

In studies of effects of low temperatures, young larvae were less tolerant and 5- to 7-day old pupae were more tolerant than mature larvae. Adults exposed to low temperatures showed greatest tolerance at 1 to 2 hours of age, as compared with those 1 to 8 days of age. Some abnormal adults were produced when pupae were held at low temperature.

6. House Flies. At Gainesville, Fla., various methods of tagging house flies have been evaluated and developed to study their biology, dispersion, and behavior. An individual male may attempt to copulate with a female at least 6 times in a day. On isolated Grand Turk Island tagged house flies dispersed in all directions from privies where they were released. In some cases flies remained around the privies for 1 or 2 days; in others they dispersed within a day. Using tagged flies it was shown that grid counts in buildings on Grand Turk represented approximately 1 to 10% of the fly population. In another study the ratio of untagged males to untagged females in the buildings was 1.5:1. The total number of flies on the island was somewhere between 200,000 and 300,000. This number of flies represents survivors of the chemosterilant bait applications which are being made on the island.

Eclosion from the pupal stage of house flies exhibited circadian rhythm. A postulate has been made that eclosion begins 12 hours after the onset of darkness. However, this response can be modified by the light regime of the parent generation, but is not obscured in toto. Preliminary data indicate that house fly susceptibility to DDT and diazinon also exhibited a circadian rhythm.

In Oregon, studies on the physiology and biology of house flies were continued. A physiological mutant strain of the house fly was isolated in which the majority of females emerge before any males appear. Strains such as this one should prove useful in biological and chemosterilization studies.

Further characterizations have been made of enzymes responsible for organophosphate resistance in house flies. The mutant aliesterase responsible for organophosphate resistance is a simple esterase. Activity of the enzyme was not affected by any of a number of metal ions or by chelating agents. Using centrifugation techniques, 50-fold purification has been achieved. Breakdown of organophosphorus insecticides by the partially-purified enzyme is inhibited by organophosphate synergists such as DEF. Studies utilizing disc electrophoresis have shown distinct differences in proteins and esterases between several susceptible and insecticide resistant strains. Evidence for allelism of the genes controlling resistance to two organophosphorus insecticides has been obtained. Also research indicated that the major genes responsible for malathion, parathion, DDT, and

Isolan resistance in house flies are all carried on the same chromosome and that genes for resistance to parathion and malathion are alleles.

7. Cattle Grubs. In Texas studies were continued to develop an artificial medium for rearing cattle grub larvae. A medium containing 80% NCTC-109 and 20% agamma calf serum proved most satisfactory for the survival of first stage larvae removed from the gullets of cattle. Survival was best in culture flasks held under near-anaerobic conditions and when the medium was changed weekly. Several larvae survived over 110 days.

8. Horse Flies and Deer Flies. Studies were continued in Mississippi on the biology of horse flies and deer flies. Adults of the spring horse fly, Hybomitra lasiophthalmus, were first observed late in March, reached a peak in mid-April and disappeared by late May. Tabanus vittiger schwardti appeared early in April and by early May small numbers of T. atratus, T. fuscicostatus and several less common species were present. These species were moderately abundant by late May. T. vittiger schwardti was abundant and T. lineola moderately abundant during June and July and T. fuscicostatus light during the same period. Small numbers of six other species were recorded.

In Mississippi an agar medium was developed for the rearing of horse fly larvae. Since the medium is translucent, the movement and development of larvae and their reactions to changes in light and temperature and to vibration are easily observed. Most of the time larvae remain in a vertical position with the terminal spiracle at the air-medium interface. However, even slight vibrations will cause the larvae to penetrate deeper into the medium. Worms or maggots placed in the medium for food are quickly located by the larvae as soon as they penetrate the medium. When approaching the surface for respiration, the larvae move backward and if entrapped air bubbles are encountered, the larvae do not move further until that air supply is exhausted. The larvae tend to seek the darkest portion of the medium when exposed to light and a temperature of 70°-80°F when there is a range of temperatures in the medium.

In Oregon snipe flies (Symphoromyia) are serious pests of animals but efforts to find their breeding areas were unsuccessful for many years. In 1963 heavy larval breeding was found in high altitudes close to water in well-drained situations, under tree trunks and in clumps of blackberry cane. Most of the larvae matured in late June but adults began emerging in mid-June. Adults were very annoying by early July.

9. Lice. In Oregon louse populations (Bovicola bovis and Linognathus vituli) increased during the summer months on cattle stanchioned in open sunlight. The increases were lower than those noted in previous tests on cattle confined in covered stanchions. When cattle were released to pasture very few lice were evident after 3 weeks. As in earlier studies it was concluded that self-grooming by cattle was largely responsible for the prevention of build-ups of louse populations in the summer months. The ratio of

males to females of B. bovis ranged from 1:16-1:20, which confirms previous observations that this species can reproduce parthenogenetically.

10. Ticks. In Texas observations were made on the seasonal occurrence of four species of ticks as follows: Lone star tick--Light infestations on cattle in January gradually increasing to heavy infestations throughout April, May, June, and July, declining in August and virtually non-existent by late September. Winter tick--Light infestations on cattle in November gradually increasing to moderate to heavy infestations in December, declining during January and February and non-existent by March. Spinose ear tick--Moderately abundant in ears of cattle from November through March, and abundant from April through October.

B. Insecticidal and Sanitation Control

1. Mosquitoes. Studies were continued at Gainesville, Fla., on the development of insecticides for the control of mosquitoes. These studies included screening of candidate chemicals against mosquito larvae and adults and laboratory and field evaluation of promising materials. In screening tests with Anopheles quadrimaculatus larvae, 70 of 226 compounds were considered effective enough to warrant further evaluation. In screening tests with Aedes taeniorhynchus adults 28 of 174 compounds were equal to or more effective than the standard insecticide, malathion.

Field tests on airplane spray applications of Bayer 41831, Bayer 39007, and malathion as water emulsions or oil solutions for adult mosquito control were conducted. Bayer 41831 and Bayer 39007 reduced the adult population of Aedes taeniorhynchus and A. sollicitans by 99% at an application rate of 0.1 lb/acre and were more effective than malathion at the same application rate.

Testing of compounds to determine their systemic toxicity against mosquitoes has been continued. Thirty-five compounds previously tested for systemic action in rabbits against body lice, were evaluated in rabbits against Aedes aegypti mosquitoes. Three of these materials (Bayer 30468, Hercules 7845-C and Rhodia R.P. 9895) caused complete mortality to at least one lot of mosquitoes fed within 5 hours after treatment (25 to 100 mg/kg) without noticeably affecting the rabbits.

At Corvallis, Oreg., fenthion applied as granular formulations as a mosquito larvicide in log ponds was effective for 7-11 days. In laboratory tests, a series of unsymmetrical esters of phosphoric acid contained materials highly effective as synergists for malathion against resistant mosquitoes.

At Fresno, Calif., in field tests against Culex p. quinquefasciatus, dichlorvos (30%) resin cylinders were lethal to larvae and adults at distances of 2 to 5 feet.

2. Stable flies. At Gainesville, Fla., 141 compounds were evaluated as stable fly larvicides. The most outstanding materials were ethyl dichlorvos,

Shell Compound 4072, Bayer 25141, and dichlorvos with LC-50's ranging from 0.21 ppm to 0.64 ppm. Other highly effective materials were phorate, Bayer 24498, Bayer 30237, Bayer 29952, Monsanto CP-7394, Monsanto CP-10613, Bayer 30750, Bayer 39007, Shell SD-8447, Shell SD-8949, Shell SD-8972, dimethoate, Ciodrin, and Bayer 22492, and Shell SD-8988, all of which had LC-50's ranging from 0.81 ppm to 1.65 ppm.

Six of the promising larvicides were tested against natural populations of stable fly larvae at dosages of 450 and 45 mg/ft² of surface. At 45 mg/ft² Bayer 39007 produced 97% to 100% control for 21 days and 94% on the 35th day; Bayer 25141 gave 99% to 100% control for 14 days but only 80% by the 21st day; dimethoate gave poor initial control (71% and 62% after 1 and 7 days) but complete control on the 14th and 21st days; and Ciodrin, dichlorvos, and trichlorfon produced 98% to 99% control within 24 hours after application but were losing effectiveness rapidly by the 7th day. Control after 35 days at both dosage levels was generally low and erratic on all the plots.

Ninety chemicals were evaluated as stable fly adulticides. Forty-eight of the compounds gave at least 80% mortality after 24 hours at a concentration of 0.25%. The most outstanding materials were Ciodrin, Pyrolan, Bayer 39007, Bayer 22684, Shell SD-4092, and Shell SD-3423 with LC-50's ranging from 0.005% to 0.029%. Other outstanding adulticides were Telodrin, Shell SD-3959, Famophos, and Bayer 37341, all of which gave LC-50's ranging from 0.032% to 0.045%.

In Texas, 177 compounds were screened in spot tests on cattle for repellency and toxicity against the stable fly. Of these materials, 4 were class IV repellents at 5.0% and 7 were Class IV toxicants at 0.25%. The outstanding repellents were ENT Nos. 15029, 27194, 27195, and 27196. The outstanding toxicants were ENT Nos. 17021, 25545, 25841, 25842, 25865, 27122, and 27163.

3. Horn Fly. In field tests in Texas, conventional spray applications of 0.05% of Shell Compound 4072 controlled horn flies for 7 days whereas sprays of 0.1-0.15% were effective for 15-22 days. In Mississippi, Shell Compound 4072 at the same concentrations was effective only about half as long as in Texas, presumably because of heavy rainfall and high humidity. Daily spraying with as little as 0.025% pyrethrins plus 0.125% piperonyl butoxide maintained excellent control of horn flies. Excellent control of horn flies was obtained in hot weather with 5% dusts of carbaryl, ronnel, dioxathion, coumaphos and methoxychlor placed in bags under cattle shelters. In cloudy, cool weather control was poor as cattle did not use the shelters.

In Texas, 7 compounds were tested as feed additives for the control of horn fly larval breeding in manure. ENT-25673 at 1 mg/kg and Famophos at 5 mg/kg daily were the only compounds which prevented larval survival. The pathogen, Bacillus thuringiensis, fed at a rate of 4 grams daily, inhibited horn fly larval development.

4. Screw-worm. Research was continued in Texas to develop more effective insecticides for controlling screw-worms affecting livestock. Fifty-two new compounds were screened for larvicidal effectiveness at 10, 1.0, and 0.1 ppm in screw-worm larval medium. None of the compounds were effective at 0.1 ppm but ENT Nos. 25612, 25780, and 25786 killed all the larvae in 24 hours at 1.0 ppm. In field tests in Mexico, cattle infested with 1-, 2-, 3-, and 4-day old screw-worm larvae were sprayed with 0.05, 0.1, 0.15, 0.2, and 0.25% Shell Compound 4072, or with 0.25% coumaphos at 2 1/2 gallons per animal. One day after treatment, no live larvae were found at 0.2 and 0.25% Shell Compound 4072, but some of the cattle were poisoned. A few larvae survived in cattle sprayed with coumaphos and lower concentrations of Shell Compound 4072. Compound 4072 at 0.1% and higher provided screw-worm control superior to 0.25% coumaphos. In a field test in New Mexico, dusts of 5% coumaphos and ronnel (Korlan) failed to afford 100% control of 1- and 2-day old screw-worm larvae in wounds on cattle, when applied by automatic duster.

5. House Flies. At Gainesville, Fla., tests were conducted with 17 emulsions against natural infestations of house fly larvae in manure at application rates of 100-200 mg/ft² of the active ingredient, using 1-2 gallons of liquid spray for each 1,000 square feet of breeding area. Dicapthon caused complete elimination of larvae within 7 days in the only test run with this compound. Indications were that Stauffer N-2404, Bayer 25141, and Shell Compound 4072 also killed all larvae in one test, but Stauffer N-2404 and Bayer 25141 had little, if any, effect in a second test. In one other test at 200 mg and two at 100 mg., Shell Compound 4072 eventually reduced the larval infestation to 13%-42% of its original size. Dimethoate, Bayer 39007, and Hooker HRS-1422 also produced considerable control in all or most of the tests. The remaining compounds were relatively ineffective.

Residual tests were conducted with emulsions of various insecticides against house flies in Florida dairy barns. All were applied at 100 mg/ft². Diazinon was included as a standard. The diazinon treatment failed after 1 day in the first test. In the second test, the reduction ranged from 78% to 90% for 5 days, after which the treatment failed. Dimethoate ranged from 83% to 94% for 8 days in one test, and from 91% to 96% for 3 days in the second test, after which the treatments became ineffective. Ciodrin gave reductions from 70% to 90% for 1 week. Fenthion ranged from 80% to 85% control for 5 days in one test, and failed after 1 day in the second. Bayer 39007 exhibited a reduction of 75% the first day, 68% the second, and was ineffective on the 3rd day after application. Endosulfan was ineffective in the first test and failed after 1 day in the second. Shell Compound 4072 was ineffective at 1 day.

At Gainesville, Fla., selected toxicants were tested as contact sprays against house flies of the regular (susceptible) and/or Cradson (multi-resistant) colonies. Dimethoate was the most effective against both colonies. Diazinon and ronnel were superior to the malathion standard against both colonies, but diazinon was indicated to be the better of the two against

the regular colony and slightly less effective than ronnel against the Cradson colony. Hercules 9326 and ENT-27160 compared favorably with the standard malathion against the regular colony flies.

At Corvallis, Oreg., research has been continued on the development of synergists for overcoming house fly resistance to organophosphorus insecticides. Tests with ethyl, propyl and butyl DEF showed that propyl DEF is an effective synergist for parathion against parathion-resistant house flies. A series of unsymmetrical esters of phosphoric acid contained materials highly effective as synergists for malathion against resistant house flies. In addition, various dialkyl analogs of parathion and malathion were effective synergists for the parent compounds. House flies selected for resistance with synergized malathion became resistant more slowly than flies selected with malathion only. Malathion resistant house flies degraded C^{14} malathion 2 to 14 times and excreted metabolites 2 to 4 times more rapidly than susceptible flies. Salithion was effective as an insecticide against susceptible and organophosphorus resistant house flies and was also effective as a synergist for parathion against resistant flies.

6. Cattle Grubs and Other Bots. Research was continued in Texas and Oregon to develop more effective insecticides for the control of cattle grubs and other bots affecting livestock. In Texas, 64 new compounds were screened for systemic action by giving them orally (O) or subcutaneously (SC) at several dosages to guinea pigs infested with larvae of Cochliomyia macellaria and Phormia regina. Eleven materials showed systemic action in one or both types of administration.

In Texas, field tests were conducted on Wyoming cattle to evaluate the systemic effectiveness of six compounds against H. lineatum and H. bovis. ENT-25658 as a 0.5% spray and ENT-26613 in feed at 7.5 mg/kg for 10 days gave 100 and 99% control of both species of grubs, respectively. A single capsule administration of 15 mg/kg of ENT-25832 was 89% effective. The other three materials were relatively ineffective.

In Texas, tests were made on 8 herds of cattle to determine the effectiveness of two applications of 0.25% coumaphos against cattle grubs (H. lineatum). Excellent control (86-100%) was obtained in 4 of 6 herds treated with conventional sprays and in two herds treated by a spray dip machine.

Also in Texas tests were conducted on small numbers of cattle (2-4) with a number of compounds that had shown promise in screening tests and with older effective materials administered in different ways. ENT-25673 at 1 mg/kg; ENT-25684 at 2.5 mg/kg, and Famophos at 5 mg/kg administered for 10 days in feed gave 100% control of cattle grubs and Imidan at 5 mg/kg gave 96% control. Trichlorfon at 1 mg/kg and fenthion at 0.5 mg/kg for 10 days afforded 88% control of grubs. Imidan and trichlorfon gave 99% control when applied at 0.25% in sprays at 1 gallon per animal. As pour-ons, 16.7% Famophos at 100 ml, 2% coumaphos at 250 ml, 2% Imidan at 65 ml and 2%

ENT-25673 at 250 ml/animal gave 100, 99, 98, and 94% control, respectively. Oil pour-ons gave significantly better results than emulsions.

In Oregon, extensive tests were run to compare the effectiveness of different volumes and different rates of application of several proven systemics as pour-ons for the control of cattle grubs. The materials tested and the amounts that gave effective control of grubs (average over 90%) were as follows: coumaphos, 4%, 97% control; trichlorophon 8%, 99% control; fenthion 1%, 94% control; and Ruelene, 8%, 100% control. In comparative tests as sprays on several herds, one application of coumaphos at 0.5% gave 93-100% control of grubs. Single applications of 0.375% and 0.25% gave 93-98 and 82-91% control, respectively, whereas two sprays of 0.25% a month apart were 93-98% effective.

In Oregon, tests were conducted to compare the effectiveness of coumaphos applied as pour-on and brush-on treatments to different areas of the body. Back brush-on and pour-on treatments were equally effective (94-98% control). Brush-ons on the escutcheon and brisket were moderately effective (81-88% control) but neck and belly applications were relatively ineffective.

7. Horse Flies and Deer Flies. Field tests were conducted to evaluate the effectiveness of 16 materials in protecting cattle from horse flies. One material (ENT-25624) was almost completely effective for 8 hours and provided a high degree of protection for 32 hours but was ineffective after 48 hours. None of the other materials were highly effective after 6-8 hours.

8. Lice. In Mississippi, 9 promising insecticides were evaluated by the "spot-test" method against cattle lice. Five of the insecticides gave 100% control of existing infestations but none showed residual effectiveness beyond 7-9 days. In feeding tests, menazon at 10-15 mg/kg daily for 3 days gave over 95% control of motile lice. Continuous feeding at this rate might eliminate louse infestations.

9. Ticks. In field tests in Texas conventional sprays of 0.1% Shell Compound 4072, 0.75% Dowco 175 and 0.5% toxaphene (standard) were equally effective in controlling the lone star tick. Conventional spray applications of 1 gallon of 0.5% toxaphene were no more effective than 0.38 gallons applied by a spray-dip machine. None of the treatments prevented reinfestations longer than a week or 10 days.

In field tests against the winter tick, toxaphene at 0.5%, Dowco 175 at 0.75% and Ciodrin at 0.3% gave equally effective immediate control. In comparative tests, applications of 0.5% toxaphene as conventional sprays (1 gal/animal) and by spray-dip machine (0.38 gal/animal) were equally effective.

Field tests against Dermacentor andersoni on cattle were made in Wyoming with recommended sprays (1 gallon per head) of toxaphene (0.5%), coumaphos (0.5%), and dioxathion (0.125%). Toxaphene was also tested alone and in combination with lanolin, Aroclor 5460 (ENT-2589), and other potential extenders at 5%, a dosage which is ten times the recommended toxaphene concentration. However,

only 378 ml (instead of 1 gallon) were used and the material sprayed only on the lower neck and brisket area, where the ticks concentrated. With the standard treatments, tick reductions for toxaphene, dioxathion, and coumaphos were 55, 71, and 91% respectively, after 7 days, and 21, 23, and 57% after 14 days. Toxaphene alone at 5% (378 ml) showed 98, 86, and 20% reductions after 7, 14, and 21 days, respectively. Toxaphene plus four extenders gave reductions of 98-100, 86-97, and 4-50% after 7, 14, and 21 days, respectively. Results of these tests indicated that toxaphene is more effective than either dioxathion or coumaphos and also that concentrated sprays of toxaphene with additives applied to a limited area on cattle provide greater residual effects than conventional sprays.

In tests in Mexico, sprays of 0.1% and higher of Shell Compound 4072 gave 100% kill of all ticks (Boophilus microplus and Amblyomma cajennense). Sprays of 0.25% coumaphos gave 100% kill of males but were slightly less effective against female ticks. Some molting nymphs survived all treatments and became adults. A few eggs were laid by 1 of 3 females from cattle treated with 0.05% Shell Compound 4072 but none hatched. One of 19 females from cattle sprayed with 0.25% coumaphos laid a few eggs but larval ticks did not emerge.

In Texas, 19 formulations of 16 insecticides were evaluated for control of the spinose ear tick in cattle. All of the materials and formulations gave excellent immediate control of ear ticks. However, after one month the most effective treatments were coumaphos, 5% dust (93-96%) followed by 0.25% coumaphos spray (77-81%). The only other treatment showing over 50% control after one month was 0.1% Shell Compound 4072 in a spray.

C. Insecticide Residue Determinations

1. Residue studies: In Texas, gas chromatographic methods were perfected for the determination of Shell Compound 4072 in animal tissues and milk. Analyses of tissues of animals 7 days after being sprayed with 0.25% of Shell Compound 4072 showed residues of 0.085 ppm only in the omental fat. None could be detected after 28 days. In a cooperative experiment with the Agricultural Engineering Research Division, two cows were sprayed with Shell Compound 4072. One was sprayed once in the conventional manner with 2 quarts of 0.15% Shell Compound 4072 and the other was sprayed daily with an automatic mist sprayer that delivered 90 ml of 0.10% Shell Compound 4072. Milk samples were collected from both cows for 15 days. Milk from the cows sprayed by the conventional method contained 0.142 ppm of Shell Compound 4072 four hours after spraying, .0095 ppm at two days and less than .0003 ppm at eight days after spraying. The level of Shell Compound 4072 residue in the milk from the cows sprayed daily with the automatic mist sprayer ranged from 0.0010 to 0.0003 ppm throughout the observation period.

A method proposed by Langlois, B. E., et al. (1964. Clean up of dairy products for analysis of chlorinated insecticide residues by electroncapture chromatography. Ag. and Food Chem., Vol. 12, p. 243.) has been used with

minor modifications for the rapid extraction and clean-up of DDT from milk, followed by gas chromatographic determination. Milk samples were analyzed from two cows, one of which was sprayed once in the conventional manner with 2 quarts of 0.5% DDT and the other sprayed daily with an automatic mist sprayer that delivered 90 ml of 0.05% DDT spray. Milk samples were taken from the combined morning and evening production. The level of DDT in the milk from the cow sprayed daily with the mist sprayer during a 24-day period never exceeded 0.0045 ppm. The level of DDT in the milk from the cow sprayed once in the conventional manner reached a peak of 0.61 ppm 3 days after treatment and fell after 14 days to 0.17 ppm., where it continued through the 21st day.

Two cows were sprayed with ronnel, one in the conventional manner, once with 2 quarts of 0.5% ronnel and the other daily with 80-120 ml of an 0.2% spray. After 14 days both cows were washed and held for one week, when analysis of the milk showed that both were free of residues. The experiment was repeated on the same cows but the treatments were reversed. Milk samples were collected for 21 days and analyzed. Residues of ronnel were below the limit of sensitivity of the analytical method. The highest levels of residues found in the milk were less than 0.2 ppm 1 or 2 days after application of the 0.5% spray.

Milk samples also were analyzed from two cows using back rubbers treated with 0.5 gallon of a 2% oil solution of ronnel. The cows were forced to walk under the back rubber at least four times a day for 28 days. Milk samples were taken for analysis at intervals during 28 days after use of the rubbers began. There appeared to be no residues in the milk resulting from the use of the back rubbers.

A cooperative experiment with Agricultural Engineering Research Division was set up to study the efficiency of a new automatic spraying device developed for the application of insecticides to livestock. A calf was sprayed twice with 5% coumaphos by passing through the automatic mist spray device. Hair samples were taken from various parts of the animal's body, extracted with chloroform, and analyzed flurometrically for coumaphos. The amount of the insecticide deposited on different parts of the body varied from 2.8 to 176.7 micrograms per cm^2 and averaging 51.5 micrograms per cm^2 .

2. Toxicity Studies. Research was conducted in Texas in cooperation with veterinarians of the Animal Disease and Parasite Research Division on the acute and chronic toxicity of insecticides and other chemicals.

A study to determine the interactions of Vitamin A and phenothiazine drenches with coumaphos was reported for FY 1963. During FY 1964 studies on blood from the animals used in that study included the effects on the Vitamin E of plasma and the Vitamin A and carotene of plasma. There are no significant differences between treatment groups for Vitamin E or for carotene. Vitamin A and carotene values decreased throughout the test in all groups. Plasma Vitamin A was affected by two interactions of treatments. With contaminated

coumaphos animals fed normal diets had lower mean values than those fed additional Vitamin A, whereas those animals treated with normal coumaphos showed no differences in plasma Vitamin A, whether supplemented with A or not.

In animals treated with normal coumaphos the plasma Vitamin A was increased by drenching with phenothiazine/lead arsenate. In those cattle treated with contaminated coumaphos the plasma Vitamin A was lowered by drenching with phenothiazine/lead arsenate.

Atropine, the standard antidote for poisoning by organophosphorus compounds, acts by opposing the stimulation resulting from accumulation of acetylcholine but does nothing to treat the basic biochemical lesion, the inhibition of the essential enzyme, cholinesterase. A need for an antidote that would reactivate inhibited cholinesterase has been recognized for many years. Various oximes have been proposed and have shown beneficial action together with specificity toward both compounds and species of animal. In previous studies, the oxime dosages employed did not seem useful against coumaphos poisoning.

A new oxime, TMB-4, has been considerably more effective than previously studied oximes in preventing death and hastening recovery of coumaphos-poisoned cattle. Pralidoxime as 2-PAM chloride (Protopam chloride), in high dosages, this year gave encouraging control of coumaphos poisoning.

Although carbamate insecticides inhibit cholinesterase, as do organic phosphorus compounds, the process is by carbamylation instead of phosphorylation. Laboratory animal studies indicated that oximes such as 2-PAM intensified the action of carbaryl instead of reversing the enzyme inhibition. Phenothiazine derivatives have some potentiating effects in organic phosphorus insecticide poisoning.

Cattle were poisoned by carbaryl, then treated with 2-PAM chloride and Promazine. Clinically, the signs of intoxication were markedly increased after the administration of the two drugs, indicating a potentiating effect of one or both.

Performance standards have been established for emulsions, but not for suspensions. Analyses of dips made with coumaphos, ronnel (Korlan) and Ciodrin were performed. Ronnel performed extremely well, maintaining its concentration precisely during the dipping of 65 sheep in a 600-gallon vat. Ciodrin was a complete failure, the concentration being reduced by more than 60% by the passage of 52 sheep through a 700-gallon vat. Coumaphos showed an essentially uniform tendency to increase in concentration, indicating that sheeps' wool was selectively absorbing more water than toxicant.

The use of present insect chemosterilants for the control of insects must be restricted because of their potential hazards. Although none of these materials are approved for use, studies were continued to determine the

hazards to livestock. Previous reports have emphasized the radiomimetic effect produced by apholate, tepa, and metepa; particularly the deleterious effect upon the tissues that form white blood cells.

Further studies have shown a second effect, teratogenesis - that is, the production of monstrosities and defects in the young of animals and birds. Incubating chicken eggs injected with the chemosterilants--apholate, tepa, or metepa at various times showed a disconcerting number of defective chicks. Defects included shortened upper or lower beaks, crossed-beaks, absence of legs, curled and fused toes, herniation of the brain, lack of eyes, schistosomus, and growth retardation. At high dosages the embryos died or did not begin development.

In further tests with chemosterilants Jersey heifers were selected and divided into groups for treatment and controls. All were observed for 3 months to establish estrous cycles, then the principals were fed apholate daily at a dosage of 1.0 mg/kg. No effect of apholate upon the estrous cycle of the heifers was apparent at the end of 7 months. The heifers were then placed with a Hereford bull for breeding, the apholate feeding continuing at the same dosage. Effects upon implantation of the embryo and upon gestation are currently being observed.

A test was completed with a single survivor of a group of four sheep given 1.0 mg/kg of apholate daily. The test feeding was terminated after the sheep survived 759 daily doses. Principal effect of apholate on this sheep was a reduction of white blood cells and blood platelets. Recovery from these deficiencies has been very slow and is still under study.

Ewes and rams fed a dosage of 0.5 mg/kg of apholate were bred during the feeding period. Ovarian and testicular biopsy tissues did not show evidence of damage by apholate. The ewes lambled normally. White blood cell numbers were slightly reduced. The test was terminated after 494 daily doses had been administered.

A second study was designed to show hematologic and teratogenic (deformity producing) effects that might occur with the feeding of apholate. Rams and ewes were selected, placed on diets containing a dosage of 1.0 mg/kg of apholate and allowed to breed. Three of four test ewes, and both control ewes, delivered normal lambs. One test ewe delivered a deformed lamb.

The deformed lamb showed a lack of eyes and eye nerves, nose, and shortened upper jaw. There was no spleen and the liver was rudimentary in size. A mass outside the body resembled liver. The dam of this lamb had received approximately 189 daily doses of apholate at the time of conception and the lamb was delivered after 345 daily doses had been given.

The response of Brahman cattle to Ciodrin, coumaphos, dioxathion and Shell Compound 4072 was compared to the responses of cattle of Hereford or other European breeding. Each of the four compounds produced a different result.

Coumaphos and Ciodrin produced greater blood cholinesterase depression in Brahman cattle than in cattle of other breeding; Shell Compound 4072 and dioxathion had just the opposite effect.

Research has continued on the treatment of animals poisoned by organic phosphorus compounds. Various oximes were studied for their effectiveness alone or in combination with atropine. Pralidoxime chloride (Protopam chloride) showed good effectiveness alone and in combination with atropine, particularly when the dosage of pralidoxime was kept high and repeated. TMB-4, a relatively new compound, gave good results in the treatment of coumaphos poisoning, the most difficult, usually, to control.

D. Biological Control

1. Mosquitoes. Cooperative studies in California have been conducted on biological control agents for mosquito larvae. Many larvae of Aedes ventrovittis and A. hexodontus and a few larvae of Aedes cataphylla and Culex tarsalis infected with microsporidia were collected in June, 1963 near Tenaya Lake in Yosemite National Park. Although infected larvae of some species were relatively abundant, the infected portion of the population was estimated at less than 1%.

Three of 21 lots of Orthopodomyia californica larvae collected in November, 1963 possessed the flagellate, Crithidia fasciculata which represents the first parasite noted in this remarkably parasite-free mosquito.

An epizootic of possibly a microsporidian (not of the genus Thelohania) was observed in several large swales in which Aedes ventrovittis larvae were present in large numbers. Many dead larvae were present and most larvae were visibly affected. Some larvae were pupating but in the laboratory most pupae died or the adults failed to emerge. Identification of the pathogen and its relationship with the host is being undertaken.

A bacterium (Bacillus sphaericus Neide) prepared by the Bioferm Corporation has been evaluated with success against many mosquito species in laboratory tests. Limited field trials against mosquito larvae breeding (Culiseta incidens, C. Peus and Aedes sierrensis) in rock and tree holes were conducted by treating these with the bacteria. Water temperature ranged from 38° - 48° F. No effect of the bacteria on the larval population was noted. It is possible that the cool temperature prevented build up of the bacteria.

2. Horse Flies and Deer Flies. In Mississippi collections of tabanid larvae in typical habitats showed about 19% of the larvae to be infected with a species of Microsporidia. Healthy larvae readily became infected after feeding on diseased larvae. The organism concentrated in the fatty tissues but also invaded the muscle and salivary glands. Muscles became nonfunctional when heavily infested.

E. Insect Sterility, Attractants and Other New Approaches to Control.

1. Mosquitoes. Studies on chemosterilization of mosquitoes were continued at Gainesville, Fla. Twenty-two compounds were tested as sterilants against larvae of Aedes aegypti. Of these compounds only two caused sterility. In other tests hempa at 50 to 100 ppm gave 99% sterility; ENT-50664 caused complete sterility in the few adults that survived the 5 ppm treatment. Feeding adult aegypti mosquitoes with seven candidate chemosterilants showed three of these to be highly effective in causing sterility in this species.

Aedes aegypti larvae and adults were treated with apholate and tepa to determine if the males would recover fertility after successive matings. Recovery of fertility was almost complete by the 4th mating with males treated as larvae with apholate. Males treated as larvae with tepa showed less recovery of fertility. There was no indication of recovery of fertility in males treated as adults with residues of tepa.

Tests with sterile males and normal females of Aedes aegypti indicated that multiple matings of females with successful sperm transfer do not generally occur. However, when the males were sterilized as larvae multiple mating of females did occur, indicating an inability of these sterile males to satisfy the sperm complement necessary in the female spermathecae to inhibit subsequent matings.

Studies with Aedes aegypti mosquitoes and the chemosterilant, apholate, were conducted to determine if resistance to the sterilizing action of this compound could be developed through selection with sub-sterilizing dosages. Selections were made by exposing larvae in treated water. Two colonies of aegypti selected over 5 to 10 generations developed resistance to the sterilizing action of apholate. Whether resistance would develop by treating adults cannot be predicted, but results indicate the possibility of the development of resistance which must be considered in the development of chemosterilants for this and other species of insects.

In Oregon, ethylenimine, a breakdown product of tepa, at 10 ppm caused no mortality of larvae, but high mortality of emerging adults of Culex p. quinquefasciatus. A dosage of 14 ppm of tepa to a ground pool containing Culex peus prevented most adults from emerging.

In Florida research was continued on factors affecting the attraction of mosquitoes and research initiated on finding specific attractants for mosquitoes. No evidence has been found to show the presence of a chemical sex attractant in Aedes aegypti mosquitoes. Chemotactometer cages were developed to evaluate mosquito response to specific chemicals.

In Oregon, extracts of both sexes of Culex tarsalis and C. quinquefasciatus showed little if any attraction to mosquitoes of the opposite sex, though in one test an ether extract of female C. quinquefasciatus provided sufficient attraction to males of that species to warrant further testing.

2. Stable Fly. Tests at Beltsville, Md., indicated that stable flies were less attracted to light than either house flies or face flies. Daylight, blacklight BLB, and blacklight BL lamps attracted small percentages of flies in outdoor cages (16% of a test population) whereas, attraction was 43% and 53%, respectively, with house fly and face fly populations.

In Texas topical applications of tretamine at 1.0 $\mu\text{g}/\text{fly}$ reduced oviposition by stable flies and no eggs hatched. An application of 4 $\mu\text{g}/\text{fly}$ prevented oviposition.

3. Face Fly. In Nebraska a field study was initiated in April and continued to September to determine the effect of releases of sterilized males on natural face fly populations. The test area involved about 1 square mile of pasture harboring 25 cattle. The weekly releases of sterile flies averaged 1500 in April, increasing to 20,000 in July, 25,000 in August, and 30,000 in September. The early releases apparently retained the build up of face fly populations but had no control effect during the summer and early fall.

Tests at Beltsville, Md., in outdoor cages indicated that face flies were more attracted by black light than house flies or stable flies. Response of face flies to light occurred principally during periods of evening twilight. In the laboratory red (660 $\text{m}\mu$), green (550 $\text{m}\mu$), and blacklight (360 $\text{m}\mu$) radiation attracted some face flies. A comparison of blacklight and red showed 65-70% of the flies attracted to the blacklight, but only 4% to the red.

4. Horn Fly. In Texas, releases of tepa sterilized male horn flies at a ratio of 3 to 1 to normal males reduced breeding by 60%. The reduction was 16% less than expected, indicating that the sterilized males were not fully competitive. In laboratory feeding tests 5 and 10 ppm of tepa completely prevented oviposition. Feeding of 1.0 ppm reduced oviposition and only 5% of the eggs hatched.

5. Screw-worm. In Texas, 22 of 158 compounds screened as chemosterilants caused sterility in one or both sexes of screw-worm when administered as topical treatments or fed to adult screw-worm flies. Some of the compounds sterilized by both methods of administration. About 18 additional compounds were sufficiently promising in screening to warrant further testing. The sexual vigor and longevity of males sterilized with ENT-50106 or ENT-50450 were reduced but those of males treated with ENT-50716 or ENT-50842 were not affected.

Higher dosages (whether topical or oral) of the chemosterilant, metepa, are required to sterilize screw-worm flies than stable flies. This verifies conclusions drawn from 1962 studies in which screw-worm flies metabolized metepa faster than stable flies and the sterilizing dose was therefore assumed in 1962 to be higher for screw-worms than for stable flies.

When screw-worm cases occur at places more than 100 miles from the known overwintering zone, the question arises about the possibility of sterilized flies recovering from radiation effects. Special tests were therefore made with flies irradiated as 5-, 5 1/2-, and 6 1/2-day-old pupae with 6200 r. Observations of flies maintained for 22 days until 95% had died of old age showed no recovery of fertility. Cytological studies of the testes and ovaries of flies treated in this manner up to 31 days old showed a continued degeneration of both testes and ovaries, with no regeneration of germinal tissue. It seems positive, therefore, that the present method of irradiation produces permanently sterilized flies.

Futher cytological studies showed the effects of a chemosterilant, tretamine, and gamma irradiation in the screw-worms to be similar, except at the first level of meiosis. Radiation of screw-worm oocytes resulted in many chromosomal aberrations during the 1st and 2nd meiotic divisions of the newly laid eggs; treatment with tretamine, however, resulted in normal-appearing meiosis, followed by visible chromosome damage during cleavage in the embryo larva.

In Texas, approximately 90 chemicals and other materials were screened as screw-worm attractants. Of these, 10 were equal to or better than the standard liver bait and require further evaluation. One of the ten materials, ENT-26926X, was highly attractive in some tests, but failed in others. A slightly detectable flowery odor suggested the presence of an impurity, believed to be ethyl isovalerate. Ethyl isovalerate synthesized at Mission and believed to be about 66% pure, was very attractive in several laboratory and field tests. Methyl isovalerate was less attractive. Most of the other 9 promising materials were choline derivatives. Several of these were highly attractive in laboratory and preliminary field tests. An attempt was made to locate pheromones in screw-worms. There was no evidence of a pheromone that would attract males to virgin females, but there was considerable evidence that there may be a pheromone produced by males that is attractive to virgin females.

6. House Fly. Research on the development of sterilization for the control of house flies has been continued. In Florida, screening of new compounds for sterilizing activity, secondary evaluation tests in the laboratory, field experiments, and basic cytological and histological studies have been continued. Of 338 new compounds screened, 49 sterilized house flies completely at one or more concentrations when fed to adults. Two new compounds, hempa and hemel, which sterilized house flies, were of particular interest because they represent a type of chemical structure not formerly known or shown to cause sterility. In tests with hempa, males were sterilized by feeding for 3 to 5 days on 1% to 2.5% hempa, but not after feeding for only 1 to 2 days. Sterilized males contained motile sperm and transferred motile sperm to the females. Males sterilized with hempa were competitive with normal males in mating with normal females.

Tests were conducted at three poultry farms in Hernando County, Fla., to evaluate the effectiveness of two chemosterilants, metepa and apholate, and an insecticide standard, trichlorfon, for the control of house flies. The

breeding site of the house flies was in manure under the poultry cages. Semi-weekly bait treatments of 1% of metepa, apholate, or trichlorfon in a sugar-water solution were applied to these droppings. Male and female flies were collected in the poultry houses for sterility determinations. The poultry house treated with metepa bait showed little reduction in the population for the first 2 weeks of treatment. Fly populations progressively decreased for the next 3 weeks, followed by a slight rise the following 2 weeks. At this time, there was such a drastic drop in the population that within 1 week no flies or breeding could be observed. The treatments were discontinued at this time. During the next 2.5 months only three flies were observed and they may have flown in or been carried from some other area. The male sterility was usually above 90%, and female sterility was often 100%. Sterility tests were discontinued after the 7th week because of the complete lack of adults in the test area. The fly population at the poultry house treated with apholate remained at a fairly constant level for the first 6 weeks of testing, after which a decrease was observed for 2 weeks, but the population increased thereafter and remained at the early posttreatment levels until the conclusion of the test. Sterility induced in both sexes was rapid and nearly complete. The fly densities at the poultry house treated with trichlorfon decreased immediately after treatment and remained at about the same level throughout the test period.

Experiments have been continued to determine whether a dosage of a chemosterilant too low to prevent complete hatching or adult emergence in the flies to which it was administered might, by the accumulation of genetic injuries, eventually reduce or eliminate reproduction after successive generations of exposure. The experimental colony in which successive generations were fed 0.01% of apholate in the adult food, is now in the F₄₀ generation. The number of progeny from treated flies pupating in the first five generations was not substantially different from the standard, but then began to decline. Only 20% to 40% of the population were reaching the pupal stage by the 34th to 39th generations. When this colony was in the F₂₂ generation, two groups of 100 pupae each were taken from the colony and two new colonies started. These were reared on regular fly food for 6 generations. The rate of pupation still varied from about 7% to 45%.

Tests were conducted to determine the effects of metepa on the chromosomes of adult male house flies. Broken chromosomes in the metaphase stage were observed in males that had been allowed to feed for 4 days on food treated with 0.5% metepa. In some males that had fed for 4 days on treated food the chromosomes in the prophase stage appeared unbroken but they stained atypically. To determine whether gamma irradiation also produced this effect, house flies in the late pupal stage were exposed to a sterilizing dosage of 2850r from a cobalt-60 source. Twenty-four to twenty-five hours after irradiation, when the adult males were 15 to 16 hours old, the testes were removed and squash preparations were made. Chromosomal damage obtained by irradiation was strikingly similar to that produced by metepa. Fragmentation ranged from mild to severe, with atypical staining of the nuclear material.

In large outdoor cage tests at Beltsville, Md., usually 40-64%, but never over 75%, of the house flies present in the cage were attracted to the

radiant energy emitted by daylight, blacklight BLB, and blacklight BL fluorescent lamps. Most of the flies which responded were attracted during the first night of exposure.

F. Insect Vectors of Diseases

1. Anaplasmosis. Studies were continued in Mississippi and Texas, in cooperation with the Animal Disease and Parasite Research Division and veterinarians of the State experimental stations, to correlate the presence and abundance of insects and ticks with the incidence of anaplasmosis in herds of cattle. In Mississippi, tests involved the exposure of three groups of cattle to insect attack--one group continuously, one group at night (1 hour after sunset to 1 hour before sunrise), and one group in daytime (1 hour after sunrise to 1 hour before sunset). Each group was exposed daily with animals infected with anaplasmosis from June 5 to July 10. All of the continuously exposed animals developed the disease within 4-5 weeks. At least one and possibly two of the other groups also contracted the disease. However, the tests were considered inconclusive and will be repeated in an effort to obtain more precise information on the relative importance of night and day feeding insects in anaplasmosis transmission. During the period in which transmission presumably occurred in these tests the principal daytime biting species were the horse flies, T. vittiger schwardti, T. lineola and T. fuscicostatus, and the mosquito, P. confinnis. No horse flies were active at night so biting was confined to mosquitoes, principally P. confinnis and A. quadrimaculatus.

During the summer animal-baited and light traps were operated at night in the Mississippi Delta in an effort to correlate bovine anaplasmosis transmission in an area as a whole with the relative abundance and feeding activity of mosquitoes and horse flies by species. The predominant species of mosquitoes based on animal traps early in the summer were Aedes vexans (about 50%), Aedes sticticus (about 20%), Anopheles quadrimaculatus (about 10%), Culex erraticus (about 6%) and Psorophora confinnis (about 4%). Later in the summer the predominant species were A. quadrimaculatus (36%), C. erraticus (31%), Psorophora confinnis (21%) and A. vexans (8%). Mosquitoes were greatest on a tethered steer, considerably less on a steer in a trap, and least in a light trap. Over 5000 mosquitoes were estimated to have fed on the tethered steer during one 12-hour test period. The feeding of this number undoubtedly adversely affected the health and comfort of the animal.

In Texas, monthly surveys were continued to determine the identity of external parasites on infected (anaplasmosis) and clean herds of cattle. Light infestations of the lone star tick were noted in January, heavy infestations from April through July, and very few after early September. The winter tick appeared in November and was abundant during December and January, and almost nonexistent by March. The spinose ear tick was moderately abundant to abundant throughout the year. Horn flies appeared in April and were present until November. Cattle were sprayed periodically to keep the adult population at low to moderate levels. Light cattle louse

infestations were noted from October to April. As a result of prompt segregation of reactor cattle, an anaplasmosis-free herd of 75 cattle has been developed during the past several years.

At Beltsville, Md., transmission experiments and cytological studies with experimental vectors were continued in cooperation with the Animal Disease and Parasite Research Division. Colonization of the Pacific coast tick (Dermacentor occidentalis) has been accomplished and one hereditary transmission experiment has been conducted. The adults were allowed to feed on a calf during the clinical stages of anaplasmosis. The progeny of these adults were then tested on a susceptible splenectomized calf. Anaplasmosis was not transmitted; however, proof of susceptibility by challenge has not yet been completed. A single engorged Dermacentor andersoni female was received from the Department of Veterinary Science, University of Nevada. This specimen was taken from a deer that, on subinoculation of blood with a splenectomized calf, was infected with A. marginale. The larval progeny of this female were placed on a splenectomized calf for testing, but the larvae did not attach and feed and all the ticks were lost.

Cytological studies on vector species have been continued at Beltsville. Infected and non-infected D. andersoni and D. occidentalis have been prepared for electron microscopy, but tissue examinations have not been completed.

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EQUIPMENT AND BUILDINGS USED IN PRODUCING DAIRY PRODUCTS

Agricultural Engineering Research Division, ARS

Problem. Modern methods of producing livestock call for increasing use of engineering principles. More knowledge is needed about the effects of environment on animals and what structures and equipment are best suited to provide the most favorable environment. Better methods of applying pesticides to animals are needed and methods of eliminating pests without using chemicals on animals would be preferred. New and additional methods are needed for using electrical and other energy to replace costly human labor in many operations devoted to feeding and care of animals. Manure disposal, especially with confinement type operations near a metropolitan area, is becoming an increasing problem. The continuing threat of nuclear warfare demands consideration of types of building that could provide protection from fallout for livestock and their feeds and provide facilities for operation during periods of emergency. To meet present day market demand for lean-type meat, producers need objective nondestructive methods and instruments for estimating the amount of lean meat in live animals.

USDA AND COOPERATIVE PROGRAM

This is a continuing program involving engineers and architects conducting basic laboratory investigations, application of laboratory results to a production basis, and development of typical plans for livestock structures. The work is in cooperation with the Animal Husbandry, Animal Disease and Parasite, and Entomology Research Divisions of ARS, USDA, and is a contributing project to Cooperative Regional Research Projects S-49, "Genetic Methods of Improving Dairy Cattle for the South", and NE-8, "Essentials of Poultry Housing for the Northeast". Plan development work is cooperative with all the State Colleges through Regional Committees, and with the Federal Extension Service, as part of the Cooperative Farm Building Plan Exchange. The professional man-years shown in parentheses at the end of each of the following sections may include work applicable to other species of animals.

A. Dairy Cattle Engineering. Dairy cattle environmental and bio-engineering studies are conducted in a climatic laboratory at Columbia, Missouri, in cooperation with the Dairy Husbandry and Agricultural Engineering Departments of the Missouri Station. AH, ARS, serves in an advisory capacity. Field studies in a hot humid region are conducted at Tifton, Georgia, with the Georgia Coastal Plain Experiment Station and AH, ARS, cooperating. During the year the project engineer at Tifton was transferred to Davis, California, but cooperation will be maintained on an "occasional visit" basis. The influences of building arrangement, equipment, and chore routines on the amount and drudgery of dairy chores and means of improving these factors are studied in cooperation with the California Agricultural Experiment Station. Typical plans for dairy structures are developed at Beltsville, Maryland. (2.6 PMY)

B. Livestock Shades and Shelters. Shades for sheltering livestock are being studied at Tifton, Georgia, in cooperation with the Georgia Station. (0.1 PMY)

C. Reducing Pesticide Residues in Animal Products. Reduction of pesticide residues in animal products, with beef cattle receiving major attention, is studied at Kerrville, Texas, in cooperation with ENT and ADP, ARS, and the Texas Agricultural Experiment Station. (1.0 PMY)

D. Water Supply and Wastes Disposal for the farmstead are studied at College Park, Maryland, in cooperation with the Maryland Agricultural Experiment Station. Liaison is maintained with the Public Health Service, the Water Systems Council, the American Society of Agricultural Engineers, and other organizations concerned with rural sanitation. (2.2 PMY)

E. Fallout Protection work for the farmstead is conducted at Beltsville, Maryland, and selected field locations. Liaison is maintained with the Office of Civil Defense, Department of Defense, and other appropriate agencies. (1.4 PMY)

F. Fly Control by physical methods in dairy barns is studied at Beltsville, Maryland. (2.0 PMY)

G. Equipment and Control for Automatic Feeding of livestock and poultry is under development in Washington and Illinois State Experiment Stations. Work on performance characteristics of upright-silo unloaders is in cooperation with the Minnesota State Experiment Station. (2.9 PMY)

H. Performance of Milk Handling Equipment is being studied at Beltsville in cooperation with the Animal Husbandry Research Division and the Eastern Utilization Research Laboratory. (0.3 PMY)

PROGRAM OF STATE EXPERIMENT STATIONS

There is an extensive program of both basic and applied research underway at the State Agricultural Experiment Stations in an effort to provide the answers to the continuing series of questions being raised by livestock producers. Demands are being made for more information on the effects of environment on the physical well being of all classes of livestock, and the way such optimum environments can be economically achieved; on new approaches to meet the growing labor shortage; on methods to adapt existing structures and equipment for greater economy of production; and on structures and related equipment for improved efficiency of feeding and materials handling operations.

Studies are being made of the effect of environment on the health, growth, production and fertility of dairy cattle, beef cattle, poultry and swine. Equipment and systems for efficiently transporting feedstuff into and out of storages and automatically mixing and feeding complete rations are being developed.

Exploring methods for improved care and housing of farm animals with greater economy and labor efficiency are also in progress as well as investigation of ways to modify existing structures and equipment to meet present-day economic conditions.

A widespread research effort is in progress which is attempting to investigate all of the factors involved in the complicated problems concerned with disposal of farm waste materials, particularly concentrated manures resulting from confine-ment type livestock operations. The problem is most acute and the public is demanding a fast solution to this unsanitary and potentially dangerous health hazard.

Studies are conducted to obtain information on uses of electrical energy and explore new uses and test equipment. Many of the projects are concerned with the varied problems of chore labor mechanization and an expansion of the use of electricity for ventilating, heating, lighting, and cooling under the various production enterprises of today's farming operations. Development and testing of prototype specialized equipment for product collection, processing, packaging, and transport, as well as storage, loading and unloading devices, are a part of the overall program of investigations.

Approximately 45 professional man-years covering work on all animal species and poultry are devoted to these problems. Much of the research is conducted cooperatively with the Department.

PROGRESS - USDA AND COOPERATIVE PROGRAMS

A. Dairy cattle engineering

1. Increasing efficiency of operations. At Davis, California, studies to determine the effectiveness of herringbone milking parlors in reducing the labor requirement in large-scale dairy enterprises were continued in cooperation with the State Agricultural Experiment Station. Time and travel required for the milking operation were measured on 7 more layouts, for a total of 48. Analyses to date indicate a big advantage in favor of using a milking machine head at each stall position in both stall rows of a two-row parlor as unnecessary delays due to slow milking cows can be avoided by a competent milking hand. The low-level milk pipeline has also shown the distinct advantage of reducing foaming in the pipeline and rancidity in the milk.

Preparation of a manuscript on milking facility layouts covering several years' study has proceeded to the final stage. Some of the principles covered have already been used as the basis for technical papers and other publications.

2. Bio-engineering studies. Basic fundamental studies on the relationships between environment and various dairy animal health and production factors were continued in the psychroenergetic laboratory, and related facilities, at Columbia, Missouri.

Studies of acclimation of lactating Holstein cows to a hot environment were continued. A 2-year summary indicated milk production after 9 weeks of heat exposure (85° F.) was only 85 percent of that at base temperatures (65° F.), indicating inability to fully acclimate to the hot environment. The partial recovery in milk production and feed consumption was correlated with increases in heat dissipating functions and decreases in heat production to a level about 15 percent below that of the base condition (65° F., 50 percent relative humidity). Very little decrease of rectal temperature was observed to follow the initial rise during the 9-week exposure to 85° F. Glutocorticoid showed a gradual recovery during the period.

Inspired-air cooling was continued. Results of two years of investigations indicate a high degree of response, although benefits to the animals were slightly below those provided by cooling the total environment to a 65° F., 50 percent R.H. condition. For lactating Holsteins housed in an 85° F., 50 percent R.H. room environment during the first year's test, milk production expressed as a percentage of expected values at a base condition (65° F., 50 percent R.H.) averaged 73 percent when inspired-air was 85° F.; 91 percent when inspired-air was 60° F.; and 90 percent when inspired-air was 50° F. Breathing heated air (85° F.) in a cool room environment (60° F.) depressed milk production and feed consumption, while respiration rates and rectal temperatures remained essentially at "normal" levels. Results of the second year's tests have not yet been analyzed.

Physiological evaluation of dairy cows as part of a long-range evaluation of physiological responses to standardized environmental conditions, was continued at Missouri. Responses of the six individual cows tested this year were more uniform than groups tested in the prior 2 years. At the standard environmental condition of 88° F., 40 percent R.H., maximum differences among individuals were 1.9° F. rectal temperature, 23 respirations/minute, and 10 heartbeats/minute.

Heat sensitivity of lactating cows was studied with cows in a single-cow hot box designed to provide a constant 110° F. air temperature. The measure of sensitivity used is the length of time required to cause a rectal temperature rise of 2° F. This is part of a continuing study at Missouri and past data have not yet been analyzed.

Air conditioning of dairy barns is under study in Missouri. Limited data were obtained during a one-month preliminary test of an air-conditioned tie-stall barn. Two groups of 16 lactating Holsteins were used, one housed in the barn and the other in a drylot outside, on an alternate 2-

week basis. There was no marked improvement in milk production, although a slight trend favorable to the air-conditioned barn was noted. However, natural conditions averaged 1.5° F. below normal during the August run--with both maximum and minimum temperatures during the month being unseasonably moderate.

Production methods for cooling dairy cattle were studied at Tifton, Georgia, an area representing hot-humid climates. The value of shade, and shade in combination with fans and water sprays, was investigated. Two groups of nine Jersey cows were held in drylot at all times (except for milking) with these two treatments, from June 16 to August 21, 1963. Cows on the shade plus fans and sprinklers treatment experienced a daily decline in milk production of 0.185 pounds compared to 0.196 pounds for the cows on the shade only treatment. These declines are somewhat larger than those found in previous studies; however, this might be expected since the average daily milk production of 39.2 pounds for the shade only treatment and 41.0 pounds for the shade plus fans and sprinklers was considerably higher than for previous studies.

3. Plan development. At Beltsville, Maryland, special studies were made of plans for free-stall housing for dairy cattle and working drawings were developed for the construction of the stalls and for the layout of a complete dairy operation incorporating this type of housing, to meet the demand brought about by the growing acceptance of this practice. Working drawings were also prepared for an open-front, individual pen type dairy calf barn and for a tilting calf table. Also, three designs were prepared for dual-purpose fallout shelter-dairy housing structures. One of these is planned to keep construction costs as close as possible to those for a conventional stall barn. Radiation protection is provided in the stall area, milkroom and personnel quarters by earth-banked walls, heavy mow floor, location of silos, and use of stored feed and bedding. The other two are for free-stall barns intended for use with loose housing systems. Radiation protection is provided by earth bank shielding around the buildings and by using a washdown system to prevent the accumulation of fallout on the roof. This permits use of low cost pole-type construction and lightweight metal roofing. The latter two do not include personnel shelters. (The man-years for the dual-purpose structures are reported under Fallout Protection, E.)

All these plans are included in the Cooperative Farm Building Plan Exchange.

B. Livestock shades and shelters. At Tifton, Georgia, in cooperation with the Georgia Agricultural Experiment Station, a study was continued in an effort to define the best height for cattle shades in a hot, humid climate such as the Southeastern United States. Three shades, 12 by 24 feet, were erected--at heights of 6, 9, and 12 feet, each covered with galvanized metal. Black globe and shielded air temperatures were recorded on various days at the animal level.

Directional radiometer traverses were made under the 6-foot and 12-foot shades. The average black globe temperature was reduced 19.6° F. under the 6-foot shade and 17.5° F. under the 12-foot shade at an air temperature of 89.3° F. and an unshaded black globe temperature of 114.2° F. The average radiant heat load was 172.8 Btu/hr/sq.ft. under the 6-foot shade and 179.9 Btu/hr/sq.ft. under the 12-foot shade. It was concluded that the radiant heat load on animals in the Southeast is greater under high shades than under low ones, and there is no thermal comfort advantage for shades over 6 feet high. Also, it seems reasonable to expect that the amount, frequency, and type of clouds would dictate the optimum height of shades in a particular area.

C. Reducing pesticide residues in animal products

Development and testing of automatic sprayers for cattle were continued because the experimental devices offer methods of providing practical and efficient control of flies with fewer residues of insecticides in meat and milk than other spraying equipment. Work is cooperative with projects ENT-h2-1, "Development of Insecticides, Repellents, and Other Materials and Methods for the Control of Horn Flies, Stable Flies, and Face Flies" and ENT-m11-2, "Development of Methods of Analysis for Insect Control Chemicals". Laboratory tests with the experimental sprayers indicated that residues of the insecticides, D.D.T., Ronnel, and G.C. 4072, left in milk by the low volume treatments, were significantly less than those of more conventional high-volume treatments. Daily automatic sprayer treatments of 80 to 120 ml. (.02 to .03 gal.) left residues not exceeding four parts per billion in milk. Concentrations that were approximately as effective as more conventional 1/2 or 1 gallon treatments left only 0.7 to 2.5 percent as much residue.

Field tests conducted during the summer of 1963 indicated that the low-volume application of Ciodrin (Shell 4294) by the experimental sprayers provided satisfactory control of horn flies on cattle. The data verified the conclusion, reached during the previous year, that the experimental sprayers controlled horn flies as well as more conventional equipment with significantly less insecticide. Operation of the sprayers under field conditions provided detailed information useful in establishing guidelines for the design of sprayers or other equipment for the self-treatment of cattle. A number of modifications with simplified and improved design were developed.

A simplified procedure for measuring the quantity of Co-Ral on the hair of treated livestock was developed in cooperation with project ENT-m11-2. The simplified procedure was used for determining the distribution of spray on a cow treated by an experimental automatic sprayer. Preliminary analysis of the results indicated that the method was quite suitable for evaluating boom and nozzle systems.

D. Farmstead manure disposal. Laboratory and field studies are continuing in Maryland, in cooperation with the Maryland Agricultural Experiment Station, on the characteristics of animal manures that affect their handling and disposal and on developing design criteria for disposal lagoons. Laboratory work has shown that a potable, sanitary "water" can be produced from manure lagoon effluent by chemical disinfection. The process should be within the means of many farmers. Observation of soil sealing and sludge buildup rates in an operating hog manure lagoon in Maryland substantiated previous laboratory findings of 39 days sealing time in a "Manor" soil and 1 mm. per month sludge buildup. Preliminary investigation of the effects of irradiation of lagoon liquids with radio-isotopes indicated that it is apparently possible to sterilize the liquids with low-level radiation and that algae cells are rendered non-reproductive for varying periods.

The major portion of a manuscript for a publication on farm animal manure disposal was prepared.

E. Fallout protection

At Beltsville, Maryland, work continued on development of plans and guide materials for fallout protection structures to be included in the Cooperative Farm Building Plan Exchange. Eight typical plans were developed for protective structures for farm families, animals, and crops.

A conventional type stall dairy barn, with radiation protection provided in the stall area, milkroom and personnel quarters by earth-banked walls, heavy mow floors, location of silos, and use of stored feed and bedding as shielding.

Two free stall dairy barns for use with loose housing systems. Radiation protection is provided by earth-bank shielding around the building and by a wash-down system on the roof. This permits use of low-cost pole-type construction and light-weight metal roofing.

F. Physical Methods for Fly Control 1/

Physical methods for controlling flies around dairy barns are being investigated at Beltsville, Maryland, cooperatively with the Animal Husbandry and Entomology Research Divisions, ARS.

Tests of the effectiveness of various commercial lamps in attracting flies to electrocutor grids were conducted in outdoor cages. Face flies were the most effectively attracted of the three species tested. They responded to three different fluorescent lamps, daylight, blacklight BL, and blacklight BLB. This proved successful only with confined populations. Attempts to kill face

1/ See also page 40.

flies around barns with similar traps were not successful. House flies also were attracted by the same types of lamps, the percentage of a confined population attracted usually being 40-65%. Light was less attractive to stable flies than to either face flies or house flies. Observations indicated that the blood feeding schedule prior to testing greatly affects their behavior.

Suitable procedures were developed for testing the reactions of face flies to monochromatic light in a Y-chamber. Initial trials indicate greatest attraction in the blacklight ultraviolet region with progressively reduced attraction at both longer and shorter wavelengths.

G. Beef and Dairy Cattle Feeding Equipment

In Illinois, work cooperative with the University of Illinois Department of Agricultural Engineering has progressed on the automatic silo unloader control for dairy and beef cattle feeding systems. A 3-position (floating control) and 2-position control was tested at hoist speeds of 5-30 inches per minute. The 3-position control worked well at all hoist speeds. The 2-position control worked best at low hoist speed and with a current differential of .1 ampere or less. With a current differential of 1.0 ampere the discharge was erratic at all hoist speeds. The severity of variation increased as hoist speed increased.

In Minnesota the performance of electric motors for the operation of silo unloaders is being determined in cooperation with the University of Minnesota Agricultural Engineering Department.

An intensive electric motor testing program was continued during the past year. The requests for information in this area seem to be growing. The capacitor motors which the unloader industry discontinued six years ago were completely tested to give a base from which to predict required performances. Only one new repulsion-start induction-run motor was introduced during the past year while several capacitor motors were introduced. At the moment only two capacitor motors appear to have been redesigned to meet the requirements of this application. Several others are only reintroductions of general-capacitor motors of the type which proved to be unsatisfactory several years ago.

Upon completion of the laboratory tests, motors with questionable performance characteristics are placed in daily operation in a silo for six months.

Several motor manufacturers are now offering their new designs for testing under this project.

In a cooperative project with the Washington State University Agricultural Engineering Department the development of an automatic trench silo unloader has progressed. The unloader has a power requirement of 9-1/2 horsepower, operates in silos with irregular side walls, has the capacity of a vertical silo unloader and can be controlled manually or automatically. Under automatic control it cuts a 12-inch by 1-inch slice from the face of the silage at the rate of 100 pounds per minute. It can operate in all types of silage.

H. Methods of Cooling Milk on Farms

Investigations were begun at Beltsville, Maryland to determine the effect on milk quality of varying the rate of cooling provided in mechanically refrigerated farm bulk milk tanks. Each trial consisted of the four successive milkings and intervening cooling and storage periods involved in an every-other-day milk pickup system. Milk samples collected before, during, and after each milking were analyzed for components of the microflora and for lipolysis. A simple aliquot sampling device was constructed to operate in conjunction with the pipeline vacuum releaser. Portions of each sample were pasteurized and evaluated by a flavor panel immediately and following eight days refrigeration. Control of the cooling rate in the ice-bank tank was accomplished by intermittent operation of the cooling water circulating pump according to a preset cycle. Milk temperature at fixed positions away from the tank walls at four levels was continuously recorded. Ambient and equipment temperatures, electrical quantities, and operations were continuously recorded.

From a series of experiments with an ice-bank tank it appears that when the quality of incoming milk is high (standard plate count of 10,000 to 40,000) the cooling rate can be decreased considerably below the maximum capacity of the refrigeration system without resulting in an increase in the microflora. The operating conditions leading to minimum detectable bacterial multiplication were found to exist between the two conditions when cooling water was circulated $\frac{1}{2}$ minute of each 5-minute cycle and when circulated $\frac{1}{2}$ minute in each 10-minute cycle. These two cycles cooled the first milking of the trial at rates of approximately 75 and 48 Btu/hr./gal. from the end of milking to 50°F. respectively. From the beginning of milking, the milk was cooled to 50°F. in about 4 and 6 hours. In the latter situation, the bacterial population rose to 8×10^6 per ml by the end of the 48-hour period. In the range studied, changes in cooling rate did not affect either the flavor score of the milk or the acid degree value.

The relation between the temperature history of the milk and the bacterial growth rate was not constant. An initial lag, followed in the second cooling and storage period by temperature-dependent multiplication, was as expected. In the period following the third milking, however, there was an actual decrease in the bacterial population. The magnitude of this decrease varied directly with the population. During cooling and storage following the

fourth milking, the bacterial population again increased at a temperature-dependent rate. This anomalous pattern of growth will be further investigated. The bulk tank studies will be extended to include direct expansion tanks, both atmospheric and vacuum. It will also be essential to study the influence of lower initial milk quality on the minimum acceptable cooling rate.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Dairy Cattle Engineering

Agricultural Engineering Research Division. 1963. Three-stall milking plant for 20 to 40 cows. (Exchange Plan No. 5875). Miscellaneous Publication No. 937. June.

Agricultural Engineering Research Division. 1963. Hay storage and feeding shed. (Exchange Plan No. 5935). Miscellaneous Publication No. 938. July. (Also listed under B, Beef Cattle Engineering)

Agricultural Engineering Research Division. 1963. Dairy barn fallout shelter. (Exchange Plan No. 5937). Miscellaneous Publication No. 943. October.

Bond, T. E., Ota, H., Hahn, G. L., and Yeck, R. G. 1964. Environmental control for animals and plants. ASHRAE Guide, Applications Volume, Chapter 31, pp. 353-380.

Hahn, G. L., Johnson, H. D., Shanklin, M. D., and Kibler, H. H. 1963. Responses of lactating cows to inspired-air cooling in a hot environment. Journal of Animal Science 22:824.

Johnson, H. D., Ragsdale, A. D., Berry, I. L., and Shanklin, M. D. 1963. Temperature-humidity effects including influence of acclimation in feed and water consumption of Holstein cattle. Missouri Agricultural Experiment Station Research Bulletin No. 846. November.

The following cooperators' publications are the results of cooperative work and report related non-engineering phases of the research:

Bergman, R. K., and Johnson, H. D. 1963. Temperature effects on plasma cortisol of cattle. Journal of Animal Science 22:854.

Johnson, H. D., and Kibler, H. H. 1963. Temperature-humidity effects on thyromine I¹³¹ disappearance rates in cattle. Journal of Applied Physiology 18:73-76.

Lundgren, R. G., and Johnson, H. D. 1964. Effects of temperature and control feeding on ^{131}I disappearance rates of dairy cattle. Journal of Animal Science. February.

Yousef, M. K., Johnson, H. D., and Kibler, H. H. 1963. Development of helmet for indirect calorimetry system utilizing gas chromatography for cattle. Journal of Animal Science 22:867.

Livestock Shades and Shelters

Hahn, G. L., Bond, T. E., and Kelly, C. F. 1963. Relation of cooling indices to electrical energy use of air-conditioned farm structures. ASAE Transactions 6(3):241-243, 248.

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Reducing Pesticide Residues in Animal Products

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Wastes Disposal

Eby, Harry J. 1963. Poultry manure disposal. Proceedings of the National Symposium on Poultry Industry Waste Management, Lincoln, Neb. May.

Eby, Harry J. 1963. Manure disposal lagoons. ARS 42-75. June

Fallout Protection

Agricultural Engineering Research Division. 1963. Dairy barn fallout shelter. (Exchange Plan No. 5937). Miscellaneous Publication No. 943. October.

Beef and Dairy Cattle Feeding Equipment

Petersen D. R. 1964. Cutter unit for horizontal silo unloader. Transactions of the ASAE. Mar. 7(1):6-7.

Puckett, H. B. and Daum, D. R. 1964. Control system for automatic silo unloading. Agricultural Engineering Journal. Jan. 45(1):26-27, and 35.

Puckett, H. B., Daum, D. R., and Olver, E. F. 1964. Metering high-moisture corn from storage. Transactions of the ASAE. Mar. 7(1):36-37.

II. NUTRITION, CONSUMER AND INDUSTRIAL USE RESEARCH

NUTRITION AND CONSUMER USE RESEARCH

Consumer and Food Economics Research Division, ARS
Human Nutrition Research Division, ARS

Problem. The assortment and characteristics of foods available to consumers are constantly changing with the adoption of new production, processing, and marketing practices. Constantly changing also, as nutrition science advances, is our understanding of the nutritional needs of man and the manner in which these needs can best be met by food. To help meet the Department's responsibility to advise consumers on the quantity and variety of foods that will assure maximum benefit and satisfaction, research must continue on the nutritional requirements of persons of all age groups, and on the nutrient and other values of foods and on how to conserve or enhance these values in household preparation and processing. Periodic surveys of the kinds and amounts of foods consumed by different population groups and individuals also are essential for evaluation of the nutritional adequacy of diets and to give the guidance needed for effective programs in nutrition education. Information from such surveys provides assistance needed in market analyses for different commodities and in the development and evaluation of agricultural policies relating to food production, distribution, and consumer use.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program of research concerned with (1) nutritive and other consumer values of raw and processed foods as measured by chemical or physical means and by biologic response; (2) effects of household practices upon the nutritive values and inherent qualities of foods, and the development of principles and improved procedures for household food preparation, care, and preservation; (3) surveys of kinds, amounts, and costs of foods consumed by different population groups and the nutritional appraisal of diets and food supplies; and (4) development of guidance materials for nutrition programs.

The research is carried out by two divisions of the Agricultural Research Service -- the Human Nutrition and the Consumer and Food Economics Research Divisions. Most of the work is done at Beltsville and Hyattsville, Maryland; some is done under cooperative or contract arrangements with State Experiment Stations, universities, medical schools, and industry. The total Federal scientific effort devoted to research in these areas total 63.3 man-years. It is estimated that approximately 7.1 man-years is concerned with studies related to dairy products.

Human metabolic studies and the related exploratory and confirmatory studies with experimental animals and microorganisms concerned with defining human requirements for nutrients and foods are not reported on a commodity basis, though some of the work is applicable to this report. This basic nutrition research represents a total Federal effort of 26.7 professional man-years and is described in detail in the report of the Human Nutrition Research Division.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

Nutrient Value of Food

Food composition and nutritive value are most frequently related to indigenous agricultural products. Specific and locally grown raw products are being extensively evaluated for essential nutrients, especially in Hawaii and Puerto Rico. Much work is related to changes induced by growing practices, processing and storage.

The form of fats and lipids in food stuffs and the changes involved in processing and holding are receiving special attention as the role of different types of fat in human nutrition unfolds. Protein content and structure continue as active research areas.

Certain raw products are being evaluated for their significant vitamin contribution to nutrition. The effect of production and processing practices on vitamin content continues as an area of interest. Additionally, research has been directed toward the study of vitamins in food stuffs as affected by inhibitory and stimulatory factors.

The total program in this area includes 36 projects in 23 States and is comprised of 23.4 professional man-years.

Properties Related to Quality and Consumer Use of Food

In the area of food preparation, products are related to quality by some measure. Special measures characterize certain classes of products; i.e., vitamin assays, enzymatic activity, water binding capacity, and changes in structural tissues. Combinations of these are involved in the quality evaluation work reported.

Food preparation research focusing on products for home use include: Heat penetration of meats and baked products and the chemical changes involved; microwave preparation of meats, fruits and vegetables, including the chemical alterations involved; and flavor characterization in frozen and stored products by means of vapor component identification.

Many of these same factors are under study in institutional preparation where the quantities involved impose special conditions.

This portion of the program includes 52 projects in 21 States and is comprised of approximately 50.0 professional man-years. This is a partial report of the State Experiment Station programs in food science and includes work undertaken by home economics departments. For research on food and fiber utilization see reports of the Utilization Research and Development Divisions.

Food Consumption and Diet Appraisal

The State program in food consumption and dietary appraisal extends the work of the Department to other segments of the population or to geographic areas not separately identified in the nationwide studies. Currently 12 States are contributing to this program. One regional project is designed to yield information regarding food purchase and consumption patterns of families with preschool children. This group represents about one-fourth of the households in the North Central Region where the study is being made. Food habits will be evaluated in terms of the children's dietary needs. This research will provide information useful to both consumer and market interests.

The State program in this area totals 22.2 professional man-years.

PROGRESS--USDA AND COOPERATIVE PROGRAMS

A. Nutrient Value of Food

1. Tables of food composition. Research for the newly revised Agriculture Handbook No. 8 "Composition of Foods...raw, processed, prepared" has been supplemented by further research during the year and adapted to the needs of special projects.

Formulas and procedures that were used in calculating the nutritive values of 250 food items commonly prepared at home are being summarized in a publication for special users, particularly therapeutic dietitians and medical research workers. A table showing average adjustments for vitamin losses during cooking has been developed and will be included in the publication.

Selected data from revised Handbook No. 8 have been made available in decks of punched cards and magnetic tape for research workers. Available in these forms are the data from Table 1, the nutritive values for 100 grams edible portion of the foods; from Table 2, nutritive values for one pound of food as purchased; from Table 3, selected fatty acids in foods. Arrangements have been made for the sale of the cards and the tape by a private data processing firm in Washington.

Tables for the Department of Defense have been prepared on the composition of 630 food items procured by the Defense Supply Agency for feeding military personnel. Values for the composition of foods developed for Handbook No. 8 and many additional values provided by the Department of Defense were used to develop the data needed for the numerous special food products meeting military specifications.

2. Vitamins. Analyses for the vitamin B₆ content and distribution in cheeses, cereal foods, fruits, and nuts available to and as eaten by consumers are nearly completed. Manuscripts are in process. Analyses on meats and vegetables are in progress.

A fluorometric procedure for the determination of pyridoxine as pyridoxal cyanohydrin was developed. The reactions were quantitatively reproducible over a range in concentration of 1 millimicrogram to 1 microgram per milliliter. Procedures for chemical assay for pyridoxal and pyridoxamine previously had been developed in this laboratory. Present studies are to adapt chemical procedures to analyze food extracts for the three forms of vitamin B₆. The procedures are expected to provide a more constantly reliable method for measuring this vitamin. Values from the chemical procedures are being compared with values obtained by microbiological determinations for vitamin B₆ in foods.

Development of coordinated procedures for B-vitamin analyses continued with emphasis on a rapid, stable chemical method for nicotinic acid.

3. Lipids. Studies of the nutritional value of various components of milk are nearing completion. Data have been obtained on body composition of rats, at 400 days of age, fed diets composed of either (1) dried skim milk and butter oil, or (2) milk protein with various combinations of fat, as butter oil or corn oil, and carbohydrate, as lactose or cornstarch. Other data include cholesterol in serum and liver, lactic dehydrogenase in serum, and an assessment of aorta damage.

B. Properties Related to Quality and Consumer Use

1. Measuring performance of fats in cakes. The effectiveness of physical measurements in indicating differences in consumer eating quality characteristics of white cakes was calculated from data obtained in the study of the performance of fats including butter in preparation of cakes in households. Viscosity of cake batters and shear force measurements of cakes were good methods for assessing performance of fats as illustrated by high correlations with panel scores for tenderness, velvetiness, and evenness of grain of cakes. Volume of cake was also a good measure of performance of fats, whereas compressibility of cake was a rather poor one.

2. Food distribution program. An improved hand-mixing method was developed to make a stable, recombined milk beverage from USDA special purchase, non-fat dry milk, butter oil, and water for use by the Food for Peace program. Amounts of milk ranging from one quart to fifteen gallons have been mixed successfully. Stable, recombined milk beverage has been made by using a mixture of the oily fraction and the crystals of butter oil as well as by using only the oily fraction.

Revision of the publication "Quantity Recipes for Type A School Lunches" (PA 631), was completed in cooperation with the Agricultural Marketing Service and the Fish and Wildlife Service, U. S. Department of Interior. This recipe card file provides 324 quantity recipes or variations and other information needed in preparing Type A lunches in schools participating in the National School Lunch Program. Recommendations on preparing, storing, and handling a wide variety of cereal, dairy, fruit, vegetable, meat, and poultry products were updated to take into account recent research findings and technology. New recipes were laboratory tested and taste panel evaluated, and all formulas and serving yields were recalculated in line with the 1964 revision of PA-270, Food Buying Guide for Type A School Lunches.

C. Food Consumption and Diet Appraisal

1. Planning for proposed nationwide survey, households and individuals. A nationwide survey of household food consumption and of the food intake of individuals is scheduled for 1965. Plans have been developed for a survey that would provide at least 6,000 household schedules and 10,000 individual schedules in the spring of the year with smaller household samples

in each of the three succeeding seasons. The information on the week's food use to be obtained from each household is similar to that obtained in 1955, except that information on home baking practices will not be requested and information requested on home food production, home canning and home freezing will be reduced to allow interview time for questions on the food intake of individuals in the households.

In preparation for the proposed first nationwide survey of the food intake of individuals, data obtained by recall on the 1-day intake of food from nearly 550 individuals of all ages in Washington, D. C. during June and July 1963, have been studied in relation to two controversial issues that concern collection of data. The survey findings indicate that for this group: (1) the nonresponse rate on food intakes from individuals is not influenced by taking a schedule on household food consumption first in comparison to taking none, nor is it influenced by taking a schedule on food intakes from half in comparison to all individuals in the family; and (2) homemakers report the amounts of food eaten by family members in terms of their individual servings far more often than as proportions of household amounts. Tabulations of the Washington data also are useful as a pretest for tabulation of the nationwide survey.

2. Effects of food distribution programs on diets of needy families. A survey of the food consumption of more than 800 households that were not participating in the food stamp program in St. Louis was made in May and June 1964 to determine the relation between usual family food expenditures and payments required for food coupons. Homemakers were asked also why their families did not participate in the program. Results of the analysis will guide the Department in revamping the St. Louis stamp program to make it more acceptable to eligible families and yet keep it within the limits of the program. Because of interest in the nutritional quality of food consumed by low-income families, an assessment may be made later of the dietary levels of these families. This is the sixth in a series of USDA food program surveys made in cooperation with the Marketing Research Division, ERS to assist the AMS to administer the food stamp and direct distribution programs.

3. Food consumption of the rural population in Spain (PL 480 research). A survey of the food consumption of the rural population in Spain has been initiated by the Spanish Ministry of Commerce under the cooperative sponsorship of the Economic Research Service and the Agricultural Research Service, using PL 480 funds. The study will provide information needed in appraising potential markets in Spain for U. S. farm products and should yield information useful to U. S. authorities on efficient ways of improving nutrition in low-income areas. The Spanish Ministry of Commerce expects to obtain much useful information on which to base a program for improving the diets of rural families, especially through better distribution of food. Information on food consumption, income levels, and related socio-economic characteristics has been obtained from about 1,200 rural families in 6 major

regions of Spain. In summarizing the results, emphasis is being placed on (1) determining the nutritional shortages among these rural families at different income levels in the different regions, and (2) computing income elasticities for different foods as well as total food consumption.

4. Effect of socio-economic factors on food intakes of individuals. Under a cooperative agreement with the Minnesota Agricultural Experiment Station intensive analysis of data previously collected showed: 1) that intake of dairy products increased somewhat with income, and that the presence of children in the family appeared to reduce the consumption by Iowa women of milk and total dairy products in low-income but not in high-income households; and 2) that intakes of vitamins A and C from food by 9- to 11-year-old Ohio children increased with family income, and at each income level, a larger proportion of urban than farm children had food that provided recommended amounts of vitamins A and C.

5. Nutritive value of national food supply. The nutritive content of the per capita food supply is calculated each year from estimates of quantities of foods consumed (retail weight basis) as developed by the Economic Research Service. This series, which begins with the year 1909, is being completely revised to incorporate newest estimates of per capita consumption, revised food composition data from Agriculture Handbook No. 8, and new information on the nutrients added to foods by enrichment and fortification.

A survey conducted by the Bureau of the Census for the Consumer and Food Economics Research Division has provided information for the years 1957-61, on quantities of enrichment ingredients supplied to processors to fortify flour and cereal products. Through this program about one-third more thiamine, one-fifth more iron and niacin and one-tenth more riboflavin are added to the Nation's diet than would be available if foods were not enriched.

For the first time, the enrichment survey was extended to include information on the quantities of ascorbic acid and vitamins A and D added to foods, thus furnishing a base line for future surveys. Currently the amount of ascorbic acid added to foods would be enough to increase the level in the per capita food supply by 5 percent. The contribution from synthetic vitamin A is 7 percent of which 6 percent is added through margarine. Vitamin D is not at present included in nutrient estimates.

6. Household practices in home freezer management. Recording forms and questionnaires for obtaining data on management practices of urban and rural home freezer owners were pretested and necessary revisions were made in preparation for data collection among households in Fort Wayne and a nearby rural area. Information will be obtained in two seasons on the kinds, amounts, sources, prices, and turnover rates of frozen foods stored in the home. Such data will provide information needed to develop guidance materials for improved management of home freezers.

7. Development of food budgets and other basic data for food and nutrition programs. Interpretation of nutrition research findings and their application to practical problems has continued as part of an ongoing program to assist nutritionists, teachers, health workers, and other leaders concerned with applied nutrition programs or nutrition policies. Information developed under this program is provided to many groups both within and outside the Department working on practical food programs, on questions relating to nutritional requirements, food consumption, nutritional importance of specified foods, and on nutrition education. Increased emphasis has been given this year to opportunities for disseminating information to the public through TV and radio, the press, conferences, workshops, and the Department's Food and Home Fair.

Food budgets at different cost levels for individuals and families are priced quarterly for publication in Family Economics Review as a continuing service to welfare workers, extension agents, and others needing this information. For example, in June 1964, the cost of one week's food for a family of four including 2 school-aged children, was estimated to be \$24.40, \$32.80, and \$37.40, respectively, for the low-cost, moderate-cost, and liberal plans.

The food budgets published in Home Economics Research Report 20, "Family Food Plans and Food Costs," have been reexamined in the light of revisions in food composition data (Handbook 8, revised) and in recommended dietary allowances of the National Research Council. Some modification in food quantities was needed for certain individuals. This has necessitated revision of food plans and their presentation in technical and popular publications, including Agriculture Handbook 16, "Planning Food for institutions," now being readied for publication. The "Food Purchasing Guide for Group Feeding," formerly a part of Agriculture Handbook 16, is in the final stages of editing for publication as a separate handbook.

All other existing guidance materials for nutrition programs were reviewed in light of the changes in recommended dietary allowances and in food composition data. Some publications have been revised; others will be updated for the next reprinting.

Nutrition Program News, a bimonthly periodical prepared for members of State nutrition committees and other community nutrition workers provides one channel for disseminating pertinent information about Federal programs and for reporting nutrition activities in the States. Issues this year included such diverse subjects as a report of the World Food Congress held in Washington, June 1963, "Labels on food products--the protection they give," and "Nutritional fitness for teenagers." Assistance to workers in nutrition programs has been provided also through consultation and program participation by staff nutritionists.

PUBLICATIONS--USDA AND COOPERATIVE RESEARCH

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DAIRY PRODUCTS - CHEMICAL, PHYSICAL AND BACTERIOLOGICAL CHARACTERISTICS:
DEVELOPMENT OF NEW AND IMPROVED PRODUCTS AND PROCESSING METHODS
Eastern Utilization Research and Development Division, ARS

Problem. Dairying is one of the largest segments of American agriculture: dairy products represent about 13 percent of all farm cash receipts; milk production requires 140 billion feed units annually (1 unit is equivalent in feed value to 1 bushel of shelled corn); milk is a highly nutritious food. It is clear from these facts that research which succeeds in increasing the consumption of milk will have far-ranging effects in raising nutritional levels, in increasing farmers' income, and in increasing consumption of feeds. There is opportunity to increase milk consumption, for per capita consumption is currently at its lowest point in over 30 years at 603 pounds, whole milk equivalent. Current consumption in the U. S. is well below that of several foreign nations, including New Zealand, Canada, Australia, Sweden, Norway and the United Kingdom, all using more than 800 pounds per capita.

Increased consumption can result from improved quality of manufactured dairy products, from cost reductions based on improved processing technology, from the development of new products, or from any combination of these. The development of new and improved processes and products is the objective of utilization research on dairy products.

Both basic and applied research in this field are needed; applied research is the direct antecedent to the development of new products and processes and basic research provides the information which permits applied research to proceed most effectively.

Increased emphasis on basic research has been advocated by the Animal and Animal Products Research and Marketing Advisory Committee, and the National Agricultural Research Advisory Committee. Basic research is considered primarily the responsibility of public agencies which disseminate their findings for use by all.

One aspect of the problem posed by dairy products is the great need for fundamental information on the complex biophysical-chemical system which each dairy product is. The development of new products and new processing technology through applied research represents the exploitation of fundamental information. Such exploitation and development cannot continue indefinitely; the supply of fundamental information must be maintained and enlarged, and this is the purpose of basic research. The complexity of milk makes necessary the employment of several scientific disciplines in basic research on this commodity. These disciplines undertake investigations needed to identify and measure the amounts of individual chemical components present. They also seek to define the molecular structure of these components; how the molecules react; and the forces determining the course of the reactions. These studies should be intensified. Other needed investigations include: study

of the synthesis of milk; the properties of milk fat; and the factors responsible for the flavor of dairy products and the changes in flavor which occur during processing and storage.

There is also need for a vigorous and sustained program of applied research which is aimed to increase consumption of dairy products. Such a research program could stimulate consumption by development of products with increased palatability, convenience, or extended shelf life. Another opportunity is the possibility of developing new and improved processing technology which will reduce costs. Because the price elasticity of milk and milk products greater than that of most food commodities, cost reduction is an attractive avenue for increasing consumption.

Still another opportunity is the development of new milk products of low fat content, for example, a low-fat Cheddar type cheese. Such a development could alleviate problems posed by current controversy over the effect of animal fats in the diet and should provide a product attractive to diet-conscious people.

Increased milk consumption, however achieved, should have a powerful upward effect on feed consumption. Since it is estimated that milk production requires 140 billion feed units annually, a 1 percent increase in milk production would require feed equivalent to 25 million bushels of corn--the production of some 500,000 acres. If the feed were supplied by cropland pasture, more than a million acres would be needed.

It is thus manifest that utilization research leading to dairy product and process development can provide a powerful stimulus to American agriculture.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving chemists, biochemists, microbiologists, food technologists, and engineers, engaged in basic research on the composition and properties of milk, and in applied research directed to the development of new and improved dairy products and processing technology.

The Department's research facilities are located in Wyndmoor, Pennsylvania, Washington, D. C., and Beltsville, Maryland. The Federal scientific effort devoted to research in this area totals 91.9 professional man-years (p.m.y.), which includes 12.3 p.m.y. in the domestic contract and grant research program. The effort is distributed as follows:

(a) Work on flavor aspects of dairy products involves 6.0 p.m.y. at Washington. One category of the flavor studies is concerned primarily with oxidized flavor. In this connection, contract research at Oregon State University, Corvallis, deals with methodology for evaluating flavor of concentrated milks; 0.4 p.m.y. is involved. A second category in the flavor investigations deals mainly with stale flavor. In this classification, contract research at the University of Illinois, Urbana, on possible flavor improvement in aged sterile milks was terminated during the year. A third

category in flavor studies relates to flavor of cultured dairy products. In this category, contract research at the Ohio Agricultural Experiment Station, Columbus, on the source of Cheddar cheese flavor was completed during the year. A fourth category in flavor research will be concerned with studies on isolation and identification of constituents responsible for desirable flavors in butter; this is the subject of research supported by a grant at Oregon State University, Corvallis (1.2 p.m.y.). In addition, research sponsored by the Department under P.L. 480 grants is in progress at: (1) National Dairy Research Institute, Karnal, Punjab, India, on sulfur compounds in relation to flavor and stability of milk; (2) Institute of biochemistry, University of Turku, Finland, on growth-promoting factors for lactic acid bacteria in cheese; (3) Biochemical Institute, Helsinki, Finland, on dietary factors controlling flavor in milk. P. L. 480 research at the National Institute for Research in Dairying, University of Reading, England, on microorganisms in dairy products has been completed.

(b) Research on whole milk products involves 21.0 p.m.y. at Washington and Wyndmoor. The program includes fundamental and applied research on development of liquid milk concentrates (6.0 p.m.y.) and dry whole milk (15.0 p.m.y.) that will be acceptable to the consumer market in quality and storage stability. In the category of liquid concentrates, a research grant at Ohio State University, Columbus, (0.8 p.m.y.) is obtaining data on the physical state of calcium phosphate-containing casein micelles in the concentrates. A new research grant at North Carolina State University, Raleigh, (1.0 p.m.y.) will be concerned with the physical changes associated with steam injection and bubble collapse during milk concentration. In addition to the domestic research on whole milk, the Department sponsors the following under P. L. 480 grants at: (1) Technical University Berlin, Berlin, West Germany, on surface changes in fat globules of dried whole milk; (2) Centro Experimental del Frio, Madrid, Spain, on protein destabilization in frozen concentrated milk.

(c) Basic research on milk involves 33.6 p.m.y. at Washington and Wyndmoor. These long-range fundamental studies include the following subjects: structures and interactions of casein and other milk proteins; bacterial spores; structure and properties of nucleic acids; influence of genetics on structure of milk proteins (cooperative with the Animal Husbandry Research Division, ARS) heat stability of milks; milk enzymes; relation of diet of the cow to milk composition. Recently initiated contract research at the University of Minnesota, St. Paul, (0.6 p.m.y.) will investigate the heat stability problem, and recently executed contract research at the University of Maryland, College Park, (0.7 p.m.y.) will be involved with the cow diet-milk composition problem. In addition, research sponsored by the Department under P. L. 480 grants is in progress at (1) National Dairy Research Institute, Karnal, Punjab, India, on milk coagulating enzymes; (2) Indian Institute of Science, Bangalore, India, on phosphoproteins of milk; (3) National Dairy Research Institute, Karnal, Punjab, India, on the proteosepeptone fraction of milk; (4) Institut National de la Recherche Agronomique, Paris, France, on non-protein nitrogenous constituents of milk; (5) Institut National de la Recherche Agronomique, Paris, France, on proteolytic activity of rennin on casein; (6) Centre de Recherches sur les Macromolecules, Strasbourg, France,

on structure of nucleic acids in connection with the synthesis of milk proteins; (7) University of Uppsala, Sweden, on methods for purification of protein complexes applicable to milk; (8) National Institute for Research in Dairying, University of Reading, England, on studies on selected enzymes of milk; (9) Instituto Nacional de Tecnologia, Rio de Janeiro, Brazil, on a study of the active site of trypsin with objective to obtain information useful in developing new dairy products.

(d) Research on milk fat, cheese, and nonfat milk involves a total of 12.0 p.m.y. in Washington and Beltsville. A considerable number of lines of work are associated with butter and butteroil stability and flavor. Cross references to these lines of work are provided in the Report of Progress section. With the availability of 7.0 p.m.y. following termination of the radionuclides investigation at Beltsville on June 30, 1964, 4.0 p.m.y. were assigned to milk fat research as of July 1, 1964. Attempts have been made to locate a suitable contractor to carry out research on fractionation of milk fat for specific food uses; implementation will depend on allocation of funds for contracting.

Research on improved ripened cheese has involved 3.0 p.m.y. during the past year at Washington. Following closeout of the radionuclides work on June 30, 1964, 3.0 p.m.y. additional were assigned to cheese research to make a total of 6.0 p.m.y. on July 1, 1964. Contract research on Cheddar cheese is mentioned under (a)--flavor of cultured dairy products. It is anticipated that contract pilot plant research on low-fat Cheddar type cheese will be initiated in F. Y. 1965, provided funds are allocated. In addition, research sponsored by the Department under P. L. 480 grants is in progress at: (1) Kaira District Cooperative Milk Producers Union, Ltd., Anand, India, on potential use of American export dry nonfat milk in manufacture of buffalo milk cheese; (2) Institute of Dairy Industry, Warsaw, Poland, on increasing vitamin B content in cheese; (3) College of Agriculture in Olsztyn, Poland, on mechanisms in the cheese-ripening process.

Research on nonfat dry milk involves 2.0 p.m.y. at Washington. Contract research at the University of Wisconsin, Madison, (0.3 p.m.y.) is concerned with the effects of nonfat dry milk on bread yeast.

(e) Research on the identification and removal of radionuclides from milk has involved 7.0 p.m.y. at Beltsville during the past year. Work on this project was terminated June 30, 1964, and EU personnel transferred to cheese and milk fat studies. The radioactive isotopes removal studies were conducted cooperatively with the Atomic Energy Commission and the U. S. Public Health Service which provided 2.0 p.m.y. additional. A research contract with the Producers Creamery Company, Springfield, Missouri, is concerned with development of a commercial scale process for removing radioactive contamination from fluid milk. This contract, supported equally by the Eastern Division and the U. S. Public Health Service, involves 7.3 p.m.y. for the USDA effort.

(f) Pioneering research on the allergens of agricultural products involves 7.0 p.m.y. at Washington.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

Research in progress on the composition and basic chemistry of dairy products begins with work designed to determine the effect of ration and season on milk composition. It extends from basic research on milk proteins and lipids to studies dealing with the chemical compounds present in specific dairy products such as cheese. Information is sought on the physical and chemical characteristics of the various protein components of the fat globule membrane. In work aimed at determining the nature and extent of the natural variation in milk with respect to its general protein components, a protein of low molecular weight and high phosphorus content has been isolated. A major study of the size, shape, structure and interactions of milk proteins has the specific objective of determining effects of protein-protein and protein-solvent interactions and changes due to enzyme action.

In the case of milk lipids, gas-liquid chromatographic techniques are being used to determine variations in and effects of feeds on the fatty acid composition of milk. Other researches attempt to establish the extent and character of solid solutions of milk fat; the influence of physical state and dispersion of the fat on lipase activity; the influence of certain metals on development of oxidized flavor in milk and milk products; and factors determining the action of lipase enzymes, including inhibitory substances.

The microbiological program with milk and dairy products begins with study of the physiology of the various organisms and extends through their application in milk processing. Lactic cultures in current use are characterized with respect to rate and total acid production, biacetyl and CO_2 production, curd tension, protein hydrolysis and contamination with undesirable species and strains. Various environmental and nutritional factors governing the growth of typical organisms are studied in pure culture. The stimulatory effect of peptide-rich extracts for certain lactic acid bacteria are being investigated. In addition, much attention is given to factors of sanitary importance, including potentially pathogenic organisms, in handling milk products.

In studies of the heat induced changes in milk, milk and milk constituents are subjected to controlled heat treatments and the changes related to flavor are characterized. An immuno-electrophoresis method has been adopted for measuring the effect of varying heat treatments upon milk proteins. High-temperature short-time, conventional heat exchange methods and direct steam injection methods of heating are used. Particular attention is being paid to changes associated with flavor development; with flavor deterioration; and with gelation and sedimentation problems in concentrated milk.

A sizeable program of research is directed to isolation, characterization and identification of the volatile and other compounds which are responsible for the desirable and attractive flavor of milk and other dairy products. A portion of this effort is devoted to establishing the chemical nature of certain

off-flavors.

A number of studies seek to improve the quality of dairy products through improved processing procedures or development of new products. Better processing methods are sought for ice cream, dry milk products, butter, cheeses and fluid milk.

The total number of professional man years devoted to this program is 90.3.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Flavor Aspects of Dairy Products.

Investigations have continued on the flavor of milk and other dairy products. Control of oxidized flavor development is believed to have been virtually realized. Attention has recently been focused on overcoming stale flavor development, a major quality defect in sterile concentrated and dry whole milk. Another flavor study in progress is concerned with factors responsible for Cheddar cheese flavor.

1. Oxidized flavor. A line of research on factors affecting flavor and storage stability of foam-dried whole milk was completed on December 31, 1963. The effects of five factors on oxidized flavor were evaluated: oxygen level of the dried milk; heat treatment of the milk; added antioxidants; moisture level; and storage temperature. These factors were interrelated and the last four were effective only in the presence of oxygen. Foam-dried milk of acceptable beverage quality over a period of six months and longer was obtained with low-heat products stored at low temperature in a nitrogen atmosphere substantially free of oxygen. Residual oxygen in the pores of foam-spray dried milk powder can be removed with a scavenging system consisting of a catalyst pellet and 5% hydrogen added to the nitrogen pack. In the continuous vacuum foam process, the product is collected and packaged in nitrogen without ever coming in contact with air.

Flavor tests were conducted during the year on 29 sterile milks at 1:1, 2:1 and 3:1 concentrations to compare scores assigned by trained with those given by preference panels. The concentrates were obtained from six manufacturers. Both the Dairy Products Laboratory and the Oregon State University (contractor) trained panel values for total and scorch-defect scores gave rather high correlations with preference scores and could thus likely be used for predictive purposes. The total score and evaluation of scorch defect in sterile milks assigned by trained panels show general agreement with judgment of an untrained preference panel.

Work on encapsulation of milk fat as a potential means of attaining flavor stability was initiated upon the arrival of a new employee. Edible film-forming substances are being used in the attempt to produce membranes of sufficient strength and continuity to preserve milk fat quality over long-term storage and handling. Preliminary results are encouraging.

2. Stale flavor. Stale flavor of milk products is caused by at least two types of compounds--lactones, formed from precursors in the fat, and ketones, some of which are believed to originate from the fat. Stale flavor development does not require oxygen, and it occurs after only a few weeks of storage in all concentrated and dried milks made by conventional manufacturing methods. The chemical nature and origin of stale flavors are still in the early investigative stages. Gamma and delta aliphatic lactones ranging from 10 to 14 carbon atoms in molecular size are generated when milk fat is heated and stored. A series of odd carbon chain-length methyl ketones ranging from C_5 to C_{15} are also generated upon heating milk fat. These lactones and ketones are strong flavor compounds and have organoleptic significance at the parts per million level at which they occur in heated milk.

Formation of methyl ketone in butteroil has been found to be dependent on presence of moisture. Hence, it is essential that all moisture be removed from butteroil to insure storage stability. O-aminoacetophenone is another ketone isolated from dry whole milk; presence of this compound at concentrations around 0.4 p.p.b. was found to cause a grape-like flavor in stale powdered milk.

Contract research completed during the past year at the University of Illinois has demonstrated that high-temperature short-time (HTST) sterilized, aseptically packaged milk is slightly superior in storage stability to in-can sterilized milk. About 14 compounds in the classes of lactones, ketones and carboxylic acids were identified in the sterilized milks.

It has been suggested that the lactone and ketone generating potential of milk fat can be destroyed by vacuum steam-stripping the fat. The idea is that the total potential lactones and ketones are formed during the process and removed in the steam condensate. It was found in EU research that deodorization of butterfat by steam stripping prevented lactone-type flavor development and produced a fat that was essentially as good in flavor after five months' storage at 80° F. as the control fat was after 0° storage. Evaporated milks have been made with deodorized fat and with control fat. Although not acceptable for beverage purposes, the taste panel preferred the evaporated milks prepared from deodorized fat.

O-aminoacetophenone, first identified in stale dry milk as a strong flavor compound originating from the nonfat portion (perhaps from tryptophane), is now suspected of being present also in HTST liquid concentrates. Taste panel studies have been initiated on milk samples deliberately supplied with certain flavor defects by additives, by use of poor processing techniques, and/or employment of poor storage conditions. Flavor defects to be evaluated include: (1) cooked flavor; (2) heated and stored milk-fat flavors (lactones, ketones); and (3) flavors attributed to maltol and O-aminoacetophenone.

Another approach to the problem of eliminating or preventing development of lactone and ketone flavors in processed milk products would be to select milks of low lactone- and ketone-generating potential. The hydroxy and keto acid precursors of these flavor compounds are products of lipid metabolism in the

cow. Analysis of individual cow milks has indicated a wide variation in the quantities of beta keto acids present. The effects of such variables as type of feed (high grain vs. high forage), season, and individual cow metabolism are to be studied in their relationships to levels of the flavor precursors.

Research at the National Dairy Research Institute, Karnal, Punjab, India, on sulfur compounds in relation to flavor and stability of milk, supported by a P. L. 480 grant, is in the preliminary stage without significant progress yet to report. The organizational phases and instrumentation have been receiving attention.

The new research project at the Biochemical Institute, Helsinki, Finland, on dietary factors controlling the flavor of cow's milk, supported by a P. L. 480 grant, is in the preliminary stage and no findings are available yet for reporting.

3. Cultured dairy products. The discovery of the relationship between the concentration of thiamine disulfide reducing substances (TDRS) and the intensity of desired flavor in Cheddar cheese is believed to be an important contribution toward identification of specific flavor-contributing substances. Development of TDRS is adversely affected by heating the milk prior to cheesemaking. Thus, this seems to explain why cheese made from raw or mildly heated milk cures to give a better flavor than that from pasteurized milk. Greater understanding of the source of Cheddar cheese flavor should now make it possible to develop practical methods of flavor control. Work on this contract research at the Ohio Agricultural Experiment Station was completed during the past year.

Research under a P. L. 480 grant at the Institute of Biochemistry, University of Turku, Turku, Finland, is concerned with growth-promoting factors for lactic acid bacteria in cheesemaking. This project is supplying much detailed and specific information on the growth cycles of lactic acid bacteria isolated as pure cultures from various dairy products. Although these microorganisms possess very complex nutritional requirements and several strains demand unknown growth factors, it was possible to formulate a basic synthetic growth medium. This was used as a basis for exploring the antagonistic and stimulating influences of certain chemicals, vitamins, amino acids, and growth regulators. Experiments on Streptococcus thermophilus show that calcium has growth-promoting action, but serine is growth-inhibiting and antagonistic in the presence of several other amino acids. Such information will be of value in deriving maximum benefit from the use of lactic acid starter cultures in cheesemaking.

Work on the P. L. 480 research project at the National Institute for Research in Dairying, University of Reading, England, on microorganisms in dairy products was completed on May 7, 1964. Findings under this investigation of cultured dairy products were reported last year.

B. Whole Milk

1. Liquid milk concentrates. A meeting of the Evaporated Milk Association, polyphosphates manufacturers, FDA and EU representatives was held to discuss the commercial use of polyphosphates as an additive to evaporated milk (2:1 concentrate). With establishment by EU research that polyphosphates are the most effective consistency stabilizers known for HTST sterilized concentrated milk, it has become necessary to know more of the fate of polyphosphates in milk. Hydrolysis of polyphosphate in milk has been found to be different than that previously observed in aqueous media. While 3:1 concentration has been believed to be the upper practical limit, experiments are being made to explore the feasibility of concentrating sterile milk to 4:1. A program is underway to determine the optimum processing conditions for producing 4:1 liquid concentrate that may be stable during storage. A test marketing of commercially produced 3:1 milk concentrate, stabilized with a polyphosphate, is now being conducted by the University of Wisconsin. Polyphosphates are also to be considered as stabilizers for frozen dairy concentrates.

Investigations are in progress on casein micelles and peptide bonds of proteins in relation to stability of dairy concentrates. Grant-supported research at Ohio State University is obtaining data on the physical state of calcium phosphate-containing casein micelles in milk concentrates. Crystalline structures have been found in frozen 3:1 concentrates. Work has been continued on a modest scale in following the course of chemical bond formation, particularly the peptide bond, by measuring the accompanying changes in the attenuation of reflected infrared energy. This technique is being used in studying reversible sol-gel transformation in milk concentrates.

A new research grant at North Carolina State University will be concerned with the physical changes in milk and milk concentrates associated with steam injection and bubble collapse. Steam injection is a promising technique for providing ultra-high temperatures for HTST sterilization, and it now becomes necessary to examine the physical changes accompanying this type of heating. No progress has yet been made toward research findings.

Research is being done at the Centro Experimental del Frio, Madrid, Spain, under P. L. 480 grant on investigating protein destabilization in frozen concentrated milk. Studies on the effects of freezing milk under various conditions on the distribution of lactose, protein and salts in the thawed separated portions have been made. Rapid freezing by spraying milk into cold air was carried out. Much data was obtained but an improved method of making frozen concentrated milk has not been found.

2. Dry whole milk. Further progress has been made in the two pilot plant processes for preparing dry whole milk--foam-spray drying at Washington and continuous vacuum foam drying at Wyndmoor. Both products initially possess good flavor. The foam-spray dried product has greatly improved dispersibility over conventionally spray dried whole milk but surface frothing at time of reconstitution still remains somewhat of a problem. Samples of the foam-spray dried product were well received by a consumer audience at the Food and

Home Fair in Washington in April 1964. While samples of the continuous vacuum foam product have not yet been distributed, very encouraging advances have been made in storage stability and in optimization of processing variables consistent with economy in processing cost.

Further progress has been made in investigating the physico-chemical factors influencing milk fat/plasma emulsion stability. The latter is important in several ways including control of dispersibility of dried whole milk and storage stability of the dry product. The film that forms on the water surface during reconstitution of dry whole milk has been found to contain 70% fat. Data have been obtained on the antioxidant distribution between butter-oil and water at different temperatures; such information should make it possible to use antioxidants more effectively. Work has been initiated on the use of the Coulter Counter for determining particle size-frequency distribution of the fat globules in milk. Such data should be valuable since globule size is probably related to dispersibility and storage stability of dry milk.

(a) Continuous vacuum foam drying. An experimental program in development of the continuous vacuum foam drying was designed to appraise the effects of seasonal changes in milk properties and of 5 of 13 controllable variables on dryer capacity and product quality. The 5 variables first investigated are: milk concentrate viscosity, content of dispersed gas (nitrogen), phospholipids (lecithin) level, belt loading and degree of vacuum. The effects of the additional controllable variables, those representing the drying conditions, are being determined and the results are combined sequentially with results from earlier less complete experiments. Tentative conclusions reached from a single season's experience are applied during the next corresponding season in moving toward optimal operating conditions. The evolutionary development of the complex drying process has become dependent on computer analysis of accumulating data, and the development has been seriously retarded by delays in processing data from finished experimental designs.

Milk powders from the continuous vacuum process have been stored at refrigerator temperature (40° F.) and room temperature (73° F.) for more than a year; storage stability proved to be limited at 73: powders made and packaged with essentially no contact with oxygen stored well at 40° for at least one year. Addition of a phospholipid, e.g. lecithin, is necessary in order to reduce the foam persisting on the belt during the early phase of vacuum drying, when operating with summer milk. Powders made from summer milk with soya lecithin added were found to have the same flavor stability as winter milk powders made without lecithin. Limited observations suggest that there may be a worthwhile gain in flavor stability at 73° if the residual moisture content of the powder is less than 3%. This low level has been attained, without heat damage, through in-package final drying by calcium oxide. The rate of final drying is so slow, however, that this procedure is not entirely satisfactory. In the hope of achieving equivalent drying much faster, the continuous vacuum dryer has been partitioned so that the milk foam is exposed to an atmosphere of very low humidity during the last one-third of its travel on the dryer belt. The consequences of this modification are now being determined.

The laboratory investigation of milk concentrate foam stability is now being integrated more closely with the continuous drying studies on the hypothesis that foam stability as measured in laboratory tests might be used to specify a quantitative "seasonal" parameter for fluid whole milk. In this manner seasonal changes could be considered in the experimental designs as an uncontrolled but measurable variable. Milk used in the continuous drying process is sampled, concentrated and the concentrate tested for its foaming characteristics. The rate of foam subsidence thus specified is treated as an independent variable.

(b) Foam-spray drying. Research findings on factors affecting the flavor and storage stability of foam-spray dried milk were summarized earlier in this report under "Flavor Aspects of Dairy Products." Concentrated milk foamed by injection of air, nitrogen or CO_2 before spray drying yields a product of excellent flavor. Injection of liquid CO_2 into the high pressure feed line of a conventional spray dryer produced distinctive powder particles that have a very open structure and produce little frothing on reconstitution in contrast to the dried product from nitrogen foaming. The spontaneous dispersion or sinking property of the powder in water has been improved by use of CO_2 foaming but not yet to the desired level. The solubility of milk powder made by CO_2 foaming is somewhat lower than that made with air or nitrogen. It is apparent that the choice of gas used in the foam-spray dryer can radically alter the structure of the powder particles. Some study has been made of ease of reconstitution of milk powders containing additives intended to enhance wettability and sinkability. Preliminary data indicate that surface-active agents may actually have a greater influence on the solubilization of the milk protein than on the dispersion of the milk fat during reconstitution.

A method was devised to fractionate butteroil by continuous crystallization from cold acetone. The primary object is to improve the ease of dispersion of milk powders by using only the more fluid fraction instead of the entire milk fat in making the powders. A possible additional result to be looked for is the concentration of flavor (or off-flavor) precursors and flavor stabilizers in the fractions. The acetone fractionation method is simple and could be scaled up easily. It has yielded a fraction, which contains 80% of the butteroil triglycerides, that is liquid at 68°F . The unsaturated and the low molecular weight fatty acid residues are concentrated in the liquid fraction. The cholesterol content apparently can be reduced by inclusion of an additional step in the fractionation.

In addition to devising means for reducing the foaming on reconstitution, a second engineering approach to improved dry milk has been to attain better fat dispersion by homogenizing the concentrate in the high pressure line between pump and spray. Homogenization of the product at the high solids concentrations now being used was previously considered impossible but was recently achieved by use of an homogenization valve. Use of this equipment leads to a marked reduction in the amount of "free" fat in the finished powder. It results in lower processing cost and should also make commercially feasible a method of manufacturing a relatively high fat-containing powder from recombined nonfat milk and modified milk fat. There is considerable interest in

the combination of butteroil and nonfat milk for providing fluid beverage milk for human feeding under the foreign aid programs and perhaps elsewhere.

One thousand cans of freshly prepared foam spray dried whole milk were distributed to visitors at the Department's Food and Home Fair in April. Each can of milk powder was the equivalent of about 2/3 quart of milk. An evaluation card was provided with each can. The proportion of cards returned, 15 percent, was normal for this sort of consumer reaction test. Ninety-five percent of the respondents indicated that the flavor is acceptable; Eighty-eight percent were satisfied with the way the milk powder reconstitutes in cold water, although some objected to its tendency to foam.

A comparison of the keeping quality of milk powders packaged in tin cans and in flexible plastic pouches has been made. The same hydrogen plus catalyst oxygen-scavenging capability was provided in both types of containers. In the cans "low heat" milk powders stored at 40° F. have maintained flavor scores well within the range of acceptability during the 4 months' duration of the test to date. None of the plastic containers tested thus far have been sufficiently gas-tight to permit comparable keeping quality. At 80° F. the flavor scores of milk powders packaged both in cans and in pouches decreased into the nonacceptable range within two months.

Studies are being carried out on surface changes in fat globules of dried whole milk under P. L. 480 grant at the Technical University Berlin, Berlin, West Germany. Results obtained using model systems indicated that the products of the amino acid-aldehyde and protein-aldehyde interactions could be isolated and characterized using chromatographic procedures. This work together with results obtained in an extensive study of the reaction of thio-barbituric acid with carbonyls is providing a solid basis for the further study of the interactions occurring on the surface of the fat globules found in milk and dairy products.

C. Basic Research on Milk

1. Isolation of milk proteins. Improved methods have been developed for the preparation of the iron-binding proteins of milk--namely, the red protein, blood transferrin, and lactoperoxidase. The methods involved chromatography on DEAE and phosphocellulose. It was demonstrated by gel electrophoresis that the transferrin of milk and bovine blood are the same.

A modified acrylamide gel electrophoresis was developed and applied to the identification and analysis of milk protein fractions. This method is of particular value in determining the purity of milk proteins.

2. Characterization and structure of milk proteins. Two of the genetic variants of β -lactoglobulin, namely, A and B, as well as the AB form and synthetic mixtures of the two, were characterized by their solubilities in water and dilute sodium chloride. It was found that the B form was about five times more soluble than the A form and that the solubilities of samples of β -lactoglobulin from individual cow's milk of a given type do not vary significantly. Synthetic mixtures of A and B types were found to interact in all proportions giving rise to a mixed crystal form with solubility characteristics of a solid solution which was related to an average of the whey fraction of the

components. Despite the fact that the B form is five times more soluble than the A form, the ratios of the solubilities in salt solution divided by their respective solubilities in water are the same for both forms as well as mixtures of the two. This indicates that the distribution of charges, as well as the amino acid sequences, of the two forms are essentially the same.

Studies on the primary structure of α -lactalbumin were continued and the complete sequences of four larger peptic peptides were established. Work is in progress on several of the other peptides.

Photooxidation studies, which proved to be so useful for the establishment of the active sites of the hydrolytic enzymes, were resumed both on model substances and on insulin. Studies on the photooxidation of amino acids have shown that the reaction proceeds as a true enzymatic reaction if one applies various kinetic treatments as used for enzymatic reactions. It would appear that photooxidation of amino acids or proteins represents an excellent model for enzymatic reactions. Photooxidation of amino acids and proteins with methylene blue was made more specific by lowering the temperature and pH during irradiation. Thus, photooxidation of insulin under such conditions brought about chemical changes, which were confined entirely to the two histidine residues of the β -chain of insulin. Biological assays of the irradiated insulin indicate that the histidine residues are intimately associated with the hormonal activity.

Investigation of the biochemical properties of casein has continued along several lines. Attention has been turned from photooxidation of α_s - and β -caseins to corresponding studies on kappa-casein. Intensive research is in progress to determine the nature of kappa-casein. Genetic studies of variations in α - and β -caseins are now virtually complete. Studies have begun on the fat that precipitates with the casein in acid coagulation of milk. Information now being obtained may prove to be of value in accounting for breed, herd and geographical differences in the processing of milk.

3. Interactions of milk proteins and other components. Study of the interactions of milk proteins in solution has been continued with emphasis on: (1) β -lactoglobulin tetramerization; (2) characterization of variant C of β -lactoglobulin and comparison with variants A and B; (3) conformation of whole casein and the α , β and kappa fractions. Accumulation of such basic data is required if the dairy industry is to achieve eventually maximum control of milk protein behavior in processed products.

Investigation of the state of casein in whole milk concentrates has been continued with emphasis on the effects of concentration and sterilizing temperatures on the binding of calcium and phosphate by the caseinate system. When more information is available in this field, processing of improved sterilized and concentrated milk products will be facilitated.

A research contract on the reactions involved in governing heat stability of individual milks has recently been executed with the University of Minnesota. No results are yet available.

4. Heat-resistant spores. The fundamental studies on the chemistry of bacterial spores have led to cultivation methods that yield large synchronous crops of sporulating Bacillus cereus. The sporulating cells are being used in preparing dipicolinic acid-synthesizing cell extracts. Basic information on cell-free biosynthesis of dipicolinic acid should contribute to a better understanding of enzymatic activities of spores in relation to their heat resistance. This in turn may enable the processing industry to sterilize dairy products with less severe heat treatment.

5. Nucleic acids. A fundamental study of the structure, degradation, and other properties and characteristics of ribosomal nucleic acids is in a preliminary stage. RNA is a key component in the protein synthesis mechanism and greater knowledge on this subject will be of importance in genetics. Mammary gland tissue for preparation of RNA is to be supplied by the Dairy Cattle Research Branch.

6. Milk enzymes. Studies on the enzymes in milk have been continued, with emphasis on the ribonucleases. It now appears that there are three ribonucleases, designated A, B and C. Determinations of amino acid composition, carbohydrate components and structure are being carried out to obtain basic information on the ribonucleases. This type of study is also being conducted on the β -caseins, A, B and C and α_s -caseins A, B and C. Hydrolysis of kappa-casein by rennin is yielding interesting results. It is important to know more about the milk enzymes and how they affect milk components during processing.

7. Relation of the cow's diet to milk composition and flavor. A research contract at the University of Maryland on the relation of milk composition, particularly the fat content, to diet of the cow is being negotiated.

8. Basic research under P. L. 480 grant program. Fundamental studies are underway at the National Dairy Research Institute, Karnal, Punjab, India, under P. L. 480 grant on the isolation and use of milk coagulating enzymes of microbiological origin for cheese manufacture. The relative milk-clotting and proteolytic activities of selected bacteria have been determined in a search for a suitable microbial source for a substitute for rennet.

Research is in progress at the Indian Institute of Science, Bangalore, India, under a P. L. 480 grant on structural studies on phosphoproteins in milk and their behavior during processing. This project has not been in effect for any great length of time and no progress report has yet been received.

Investigations are being made on the proteose-peptone fraction of milk under P. L. 480 grant at the National Dairy Research Institute, Karnal, Punjab, India. A new colorimetric method has been devised to determine the proteose-peptone content. Using this method 150 samples of cow milk and 50 samples of buffalo milk were studied. Results indicate that no striking concentration differences exist between the proteose-peptone fractions of the milks taken from these animals.

Research is being done on nonprotein nitrogenous (NPN) constituents of milk under P. L. 480 grant at the Institut National de la Recherche Agronomique, Paris. Studies concerning the soluble NPN products resulting from the action of rennin on casein were concluded. It was established that more than 80% of this fraction could be accounted for by the peptides split from the kappa-casein. Further studies show that the increase in NPN noted on heating milk arises from the release of materials different from those released by the action of rennin on casein.

Research at the Institut National de la Recherche Agronomique, Paris, France, under a P. L. 480 grant is concerned with investigating the proteolytic activity of rennin on casein. This research is concerned with an elucidation of the enzymatic coagulation of milk to obtain basic information for use in devising improved cheesemaking processes. A pure crystalline form of rennin was reacted with the various protein components known to form the casein moiety. It was shown that the clotting action of rennin on milk depends on a reaction between the enzyme and kappa-casein. In the presence of α_s -casein, the action of rennin on kappa-casein was inhibited. The strong tendency of α_s -casein and kappa-casein to interact is believed to be significant also in the heat stability of milk and in the gelation which occurs in condensed milks on prolonged storage.

Fundamental studies underway at the Centra de Recherches sur les Macromolecules, Strasbourg, France, under P. L. 480 grant are probing the relationship between genetic factors and the synthesis of proteins to help explain why a particular kind or quality of protein is formed in a given species of plant or animal. It is envisioned that results from this and related research will have far-reaching effects on the selective breeding of crops and livestock. Since previous research indicates that structural changes in a protein's deoxyribonucleic acid (DNA) molecule dictates the formation of genetically different proteins, these workers are acquiring detailed chemical and physical data on the molecular sub-units of DNA. Substantial progress has been reported in the initial phases of the project. Techniques have been developed for separating native DNA from artificially denatured material, and for detecting compositional differences. The enzyme, spleen deoxyribonuclease, has been isolated in the pure state for the first time and has been utilized in degrading the DNA molecule into smaller units for further study.

The research at the University of Uppsala in Sweden, under a P. L. 480 grant, on methods for purifying protein complexes that would be applicable to milk is just in its beginning. Hence, there is nothing to report at present.

Studies are underway on selected enzymes of milk under P. L. 480 grant at the National Institute for Research in Dairying (University of Reading), Shinfield, Reading, England. A technique for the rapid and direct recording of small pH changes was developed and used to study the detailed kinetic properties of various enzyme systems found in milk. A study of lipase activity in milk taken from 20 cows at various stages of lactation revealed marked differences in lipase activity of milk drawn from individual cows.

Under a P. L. 480 grant at Instituto Nacional de Tecnologia, Rio de Janeiro, Brazil, studies on the purification of crystalline trypsin resulted in the separation of several enzymatically active components. Methods for these separations were developed both for analytical as well as for preparative scale. The apparent enzymatic heterogeneity of trypsin might be due to the incipient autolysis of the enzyme. Further characterization of these fractions will permit the correlation between change in biological activity and the modification of structure.

D. Milk Fat, Cheese and Nonfat Milk

1. Milk fat. Probably the most important technical problems connected with extending the utilization of butterfat are related to flavor and storage stability. Thus in the foregoing sections of this report in "Report of Progress" dealing with flavor research, research on whole milk products and basic research, many of the lines of investigation deal with butter and butter-oil. For purpose of cross reference, the following topics are discussed under "Flavor Aspects of Dairy Products": encapsulation of milk fat; lactones and ketones originating from milk fat; deodorization of butterfat by steam stripping. Under "Whole Milk" progress, the following subjects pertinent to the fat component are discussed: antioxidant distribution between butteroil and water; fractionation of butteroil; improved dispersion of butterfat in foam-spray drying of milk. Under "Basic Research on Milk," the new contract research on relationship of butterfat and other milk components to the diet of the cow is mentioned.

Many believe that butter use could be materially extended, particularly by taking advantage of its unique flavor and certain other properties, if fractions were available to provide "tailor-made" products. Hence, attempts have been made to locate a suitable contractor to carry out research on fractionation of milk fat for specific food uses. No work has been started on this project, and implementation will depend on allocation of funds for contracting.

2. Cheese. Pilot plant work in 1959-1960 on methods of improving the quality of Cheddar cheese indicated that flavor enhancement of this cheese may be obtained by controlled lipolysis. This phase of research was stopped July 1, 1960, and personnel transferred to Isotope Removal Investigations. Laboratory research continued and resulted in development of a selective medium for Leuconostoc, which provides a new means for detecting flavor-forming bacteria in lactic starters, cheese and other cultured dairy products. An important discovery, now widely used commercially, was that the destructive growth of bacteriophage in cheese starters can be prevented by phosphate-heat treatment. This latter line of work was discontinued on February 25, 1964.

It is considered by many persons engaged in research and marketing of dairy products that a flavorful cheese of good texture, relatively low in fat and high in protein, should meet with ready demand. Continued progress has been achieved on development of a laboratory procedure for producing low-fat cheese. The new low-fat cheese has only about 5% fat compared to 31% for

ordinary Cheddar. While further laboratory and pilot plant work remain to be done, results are quite promising in regard to the texture and flavor factors relative to these quality attributes in low-fat cheese made by conventional methods. It is anticipated that additional manpower can be assigned to this work after July 1, 1964, and initiation of contract pilot plant research is expected, provided funds are allocated.

Studies are being made at the Kaira District Cooperative Milk Producers Union, Ltd., Anand, India, under P. L. 480 grant on investigations of the addition of nonfat dry milk solids to buffalo milk in the manufacture of hard cheese as a means of expanding overseas markets for dry milk. This research shows that Cheddar-type cheese of satisfactory body and texture can be made from native buffalo whole milk when it is standardized with imported nonfat dry milk, but flavor development is slow and insufficient.

Research is being done at the Institute of Dairy Industry, Warsaw, Poland, under P. L. 480 grant on the development of mutant strains of molds with increased ability to synthesize vitamin B for use in improving the quality of mold-ripened cheese. Penicillium roqueforti has been found to increase the level of riboflavin, niacin, pantothenic acid and biotin in Roquefort cheese during ripening. Some of the induced mutant strains were found to be much more vitaminogenic than others.

Investigations are being carried out at the College of Agriculture in Olsztyn, Olsztyn, Poland, under P. L. 480 grant on the mechanisms of the cheese-ripening process to obtain fundamental information for use in developing methods for manufacturing improved quality cheese. An exhaustive study is being made to determine the products formed during the fermentation of fat, protein and lactose in a variety of cheeses in an attempt to establish the mechanisms of fermentation and possible relation between specific fermentation products and flavor.

3. Nonfat dry milk. Foam-spray dried nonfat milk is now being manufactured by several producers using the EU process. One of these processors has the capacity to turn out a large volume of product, and the industrial pioneers in foam-spray drying are presently engaged in developing outlets for this new type of nonfat dry milk (NFDM). Our work on development of increased food outlets for NFDM has been greatly curtailed since the death of a senior staff member about a year ago. While new product work could not be emphasized, considerable progress has been made on equipment and processing. A practical in-line homogenizing valve arrangement has been developed that will reduce equipment cost in spray drying. Development of an in-line high-solids evaporator will permit foam spray drying of 60% nonfat milk solids concentrate under conditions that are expected to avoid protein denaturation. Equipment has been installed for compressing dried milk into cubes to reduce volume markedly.

In addition to the commercial adaptation of foam-spray drying to NFDM, several large dairy products manufacturers have recently started foam-spray drying cottage cheese whey. One of these companies alone can produce 3 million

pounds of dried whey annually. Air is injected into the concentrated whey immediately before it enters the spray-drying chamber. The resulting product is free-flowing and disperses readily in contrast to conventionally dried whey which tends to be sticky, lumpy and difficult to reconstitute. Most of the cottage cheese whey has been dumped in the past. The entire cottage cheese industry has a potential to produce nearly a billion pounds of dried whey annually, worth about \$100 million, provided additional food uses can be developed for the product. It has been demonstrated that foam-spray drying is also advantageous for drying Cheddar cheese, milk chocolate mixes, ice cream mix, malted milk and other dairy products.

Bread has provided an important outlet for NFDM. This was threatened when a large fraction of the bakeries went on a short-time continuous dough mixing procedure in which little or no NFDM can be tolerated because of dough handling and loaf texture problems. The objectives of contract research at the University of Wisconsin are to determine the advantages of NFDM in dough fermentation (besides the obvious contributions to nutrition and flavor) and to search for modified conditions of fermentation and dough handling that will permit the use of NFDM in the new continuous breadmaking process. It has been demonstrated to date that nonfat milk solids greatly stimulate the yeast fermentation.

E. Radionuclides in Milk

Extensive nuclear testing, particularly in 1950-1961, made it advisable to develop practical means for removing radioactive isotopes making their way into milk through consumption of feed exposed to fallout. Laboratory and pilot plant studies on removal of radioactive contamination from milk have demonstrated that commercial scale withdrawal of radioactive strontium and cesium by an ion-exchange fixed bed process is feasible. Iodine-131 can be removed by use of an anion resin column if necessary in time of emergency, but further research is needed on removal of this particular radionuclide. Work on this project was terminated June 30, 1964. The studies on removal of radioactive isotopes have been conducted in cooperation with the Atomic Energy Commission and the United States Public Health Service.

An automatic commercial size plant for removal of Sr-90 from milk has been established under contract at Producers Creamery Company, Springfield, Missouri. Findings from the Beltsville pilot plant are being used as a basis for this commercial scale plant. Start-up of the Springfield plant was originally scheduled for March 15, 1964, but was delayed about 60 days because of slow delivery of certain valves.

Cooperative research with the U. S. Public Health Service has been extended through a memorandum of understanding to include a pilot plant study of a moving resin bed ion exchange system for removing radionuclides from milk. A moving bed contactor is being purchased and will be installed at their Southeastern Radiological Health Laboratories, Montgomery, Alabama.

F. Allergens of Agricultural Products

The objective of the milk allergens research is to identify and characterize the constituents in cow's milk that produce undesirable reactions in infant feeding and to seek practical methods of inactivating these allergens. Special laboratory facilities for a collaborative program with research workers at Jefferson Medical College, Philadelphia, were set up and investigations started. However, this collaborative program was unexpectedly terminated when urban renewal displaced and scattered the families that had been counted on to provide clinical material. Hence, negotiations will have to be developed with another medical collaborator in order to continue this study.

Studies on the immunochemical characterization of ribonuclease of cow's milk and from bovine pancreas have been completed. Ribonuclease isolated from bovine milk by the Milk Properties Laboratory was shown to be serologically identical to pancreatic ribonuclease. This suggests that ribonuclease present in cow's milk originates in the glandular tissue of the pancreas, which is considered to be a finding of considerable fundamental importance.

Castor bean allergens have been under study for the past several years. The castor bean allergen fraction designated as CB-1A is being fractionated and characterized as to antigenic and allergenic components. The immunological relationships of CB-1A, castor bean meal, and castor bean pollen are being determined. CB-1A has been found to be a complex mixture of proteins and polysaccharidic proteins with a common antigenic specificity or, at least, closely related antigenic specificities. Studies are being made on the carbohydrate-free protein mixture isolated from CB-1A and designated as CB-65A in search of structural differences that may explain immunological distinctness. The subject of castor bean allergens is extremely complex, requiring development of precise methodology for elucidation of structure and correlation with immunological behavior.

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III. MARKETING AND ECONOMIC RESEARCH

DAIRY PRODUCTS - MARKET QUALITY
Market Quality Research Division, ARSProblem.

Modern marketing practices in the dairy industry have intensified the problems of detecting inferior lots of milk and of increasing the storage life of dairy products. Several kinds of insects and mites contaminate or damage dairy products during processing, storage, and distribution. To maintain quality of these products in marketing channels, research is needed on the factors influencing keeping quality; on developing new and improved objective quality tests for bulk milk and other products; on developing safe and effective procedures for preventing or controlling insect and mite infestations; and to find improved and simplified detection methods for antibiotic and pesticide residues in dairy products.

USDA PROGRAM

There is a continuing program of basic and applied research aimed at developing new and improved methods for assessing the important quality factors in a variety of dairy products. Work on simplified methods for detecting chlorinated pesticide residues in dairy products and on the shelf-life of canned butteroil is being carried out at Beltsville, Maryland. The final write-up of results from a two-year contract with the University of California at Davis on the estimation of protein content of milk by dye-binding is nearing completion. A three-year contract with the University of Minnesota to study the quality of milk used for manufacturing purposes is nearing completion, ahead of schedule. The Federal scientific effort devoted to research in this area totals approximately 2.0 professional man-years, all in objective measurement and evaluation of quality.

There is a continuing program involving entomologists and chemists engaged in basic and applied research on the prevention of insect infestation and contamination of dairy products in the marketing channels, headquartered at Fresno, California.

The Federal effort of about one professional man-year was temporarily diverted during this reporting period to emergency research on the effects of gamma irradiation on stored-product insects, which is also pertinent to the problems of controlling insects and mites in dairy products. Much of the cross-commodity research at Savannah, Georgia, under the title, "Insect Control in Marketing Channels," is also applicable to the problems in dairy products.

Line Project MQ 1-13, dealing with developing improved insect-resistant packages for nonfat dry milk, was terminated with completion of the major objectives of the project, and the incorporation of upgraded packaging requirements in the ASCS purchase specifications.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

Research concerning market quality of dairy products is conducted under 24 projects in progress at 17 state stations. The work is part of a continuing program of research on dairy products.

Study of the factors influencing the accuracy of sampling milk is in progress. The objective of developing rapid, precise, and accurate tests for estimating the freshness of milk and for determining its butterfat or protein content and microbiological quality provides the impetus for several studies. Comparison of several rapid methods for protein determination in milk and milk products is under study with dye binding methods receiving special emphasis. One project's goal is development of a technique for rapidly determining the bacterial population of, or the extent of bacterial activity that has occurred in, raw milk and making this technique applicable for use by unskilled operators under field conditions. Another project emphasizes establishment of quality control procedures for raw milk supplies. Other related work seeks to ascertain the average value and normal range of freezing points of milk and determine the effects on freezing point of various production, environmental and processing factors.

Study of the changes in milk quality resulting from conditions associated with movements of milk to processing plants is underway. Experiments are also in progress to determine the effects of sanitation procedures on milk quality and flavor.

Insight into the causes of quality deterioration of certain dairy products such as cottage cheese, butter, ice cream and cheese is also sought. Methods of improving quality include study of controlling length of shipping time, temperature changes during storage and shipping, and general retail practice.

Other research involves improvement of the keeping quality of butter and butteroil through study of trace hydrogenation techniques and use of various antioxidants in butteroil. Factors influencing the market quality of cottage cheese are investigated. The objectives of another study include observation of the effects of composition and processing techniques on the

physical and chemical properties of ice cream and the various quality indicators of the finished product. Evaluation of the quality of and consumer reaction to milk, dry milk products, ice cream and cheeses is made to facilitate quality preservation and quality improvement in dairy products.

A total of 6.9 professional man-years is devoted to market quality research on dairy products.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Objective measurement and evaluation of quality

1. Pesticide Residues. Studies of the use of thin-layer chromatography for analysis of chlorinated pesticide residues showed that this technique is faster and more sensitive than paper chromatography while at the same time retaining the advantage of simplicity and low cost. Details of procedures were worked out using thin-layer plates prepared from alumina or silica gel with a small amount of silver nitrate to develop a dark spot under ultraviolet light in the presence of chlorinated compounds. Florisil and carbon-celite chromatographic cleanup techniques were found satisfactory for sample preparation. Some lots of florisil purchased recently contain excessive amounts of interfering materials and it will be necessary to develop a procedure for removing these before the florisil can be used for cleanup of samples.

(MQ 3-11)

2. Protein Content. The study on the estimation of protein content of milk by dye-binding is being carried out at the University of California. During the past year, statistical analysis of the data was completed and preparation of the material for publication is nearing completion. Statistical analyses showed that a better relation between dye-bound and protein can be obtained if a correction for fat content is included in the regression equation. No explanation can be made for this effect.

(MQ 3-14(c))

3. Manufacturing Milk. A survey of the quality of milk for manufacturing was carried out under contract with the University of Minnesota. (Collection and analysis of samples were completed well ahead of schedule. The data have all been transferred to punched cards for statistical analysis.) Preliminary examination of the data indicate that even with refrigerated bulk tanks, some producers are selling milk of rather poor quality, indicating lack of attention to sanitary practices. Reduction methods and direct microscopic counts tend to grade milk somewhat more leniently than the standard plate count when the proposed U.S.D.A. standards for each method are used.

(MQ 3-44)

4. Stability of Anhydrous Butterfat. Samples were put in storage in sealed cans at 32°, 70° and 100° F. Sampling after six months-storage showed little change at 32° F, some at 70° F, and more at 100° F. Chemical tests run were the peroxide value and the thiobarbituric acid test and the samples were also evaluated organoleptically. The slight deterioration observed was not sufficient even at the 100° F storage for six months to cause the samples to be unacceptable for use.

(MQ 3-49)

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Objective Measurement and Evaluation of Quality

Moats, W. A. 1964. One-step cleanup of chlorinated insecticide residues by chromatography on carbon-celite mixtures. J. Assoc. Off. Agr. Chem. 47:587.

(MQ 3-11)

MARKETING FACILITIES, EQUIPMENT, METHODS,
CONSUMER PACKAGES AND SHIPPING CONTAINERS ^{1/}
Transportation and Facilities Research Division, ARS

Problem: A survey of market milk and ice cream plants throughout the country, by the University of Illinois in 1958, shows that the equipment, work methods, and facilities of many of these plants are obsolete and the production per man-hour employed relatively low. A major factor contributing to this obsolescence is the development during the last few years of new types of equipment which can be brought under automatic control. Because of the investment required and the uncertainties of the returns they would obtain, plant operators have been reluctant to shift to automated equipment on a piecemeal basis. They also have been reluctant to build new plants because of a lack of guidelines and criteria on automated plants. Engineering layouts and operating criteria therefore are needed for automated plants to provide guides to plant operators in making the shift from their present equipment and facilities. Most dairy plants lack the technological and engineering skills necessary to plan and develop suitable plant layouts and designs, or to select the types of equipment needed and the controls necessary for full automation. Automated equipment and processes for some types of dairy plants still largely are lacking or are nonexistent.

^{1/} The work described here is part of an overall program aimed at improving market facilities and market operations. As agricultural commodities flow through marketing channels they converge with similar products, for example, meat, poultry, fish and dairy products are often handled by the same wholesaler and reach consumers through the meat and produce department of retail stores. Because of this situation, improvements in the overall marketing process can bring about benefits that affect several commodities simultaneously. The component costs of marketing have been rising rapidly and would have risen more if the results of this type of research had not been available. In the marketing of food commodities in 1963, at least \$30 billion (75% of the total food marketing bill) were expended on marketing operations that are directly affected by the research covered in the overall program. The overall program includes (1) terminal wholesale marketing planning, (2) preliminary and followup work in terminal market areas, and (3) production area and independent marketing facilities such as that described here. Terminal wholesale market planning was conducted in 7 major cities last year. Production area and independent market facilities planning involved 41 studies. For additional information see "A Summary of Current Program and Preliminary Report of Progress" dated September 30, 1964, by the Transportation and Facilities Research Division, ARS, USDA.

It costs about 8 billion dollars a year to package food products, but without shipping containers and various other types of packages it would be impossible to move farm products efficiently from the widely dispersed areas of production through our complex marketing system to millions of consumers. New or improved packages and containers must be developed and evaluated to do this job move effectively. In protecting, distributing and selling perishable agricultural commodities, packages and containers must respond to a number of marketing system changes.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program devoted to planning marketing facilities in which application is made of engineering, economic, and marketing principles. This work is concerned with structures, equipment, containers, devices, work methods, and operating methods used in marketing and transporting farm and food products from farms to consumers. The functions to which these physical elements, handling methods, and labor relate include essentially all marketing operations, especially those directly applicable to the commodities in the physical sense such as assembling, preparing for market, processing, packaging, precooling, loading, transporting, unloading, storing, warehousing, and wholesale and retail distribution. The part of the program pertaining to dairy involves agricultural engineers and dairy technologists engaged in applied research to develop improved methods, equipment, operating criteria, and plant layouts for dairy plant operators.

Current research covers the development of layouts and operating criteria, based on current technology, for automated dairy product plants. It features the use of remotely operated valves, electronic-controlled devices, and highly mechanized equipment. Work in the Hyattsville office consists of checking, organizing, and preparing for publication a series of reports prepared under contract.

The Federal effort devoted to research in this area during the fiscal year 1964 was 1.7 man-years: 0.5 man-year for direct work (this employee was enrolled at the University of Minnesota for full-time training in dairy technology and 0.8 man-years, consumer packages and shipping containers, and 0.4 man-year for program leadership.

PROGRESS - USDA AND COOPERATIVE PROGRAMS

Layouts and Operating Criteria for Automated Dairy Product Plants

At Hyattsville, Md., work was continued on the preparation for Department publication of a series of contract reports covering the development of layouts and operating criteria for different types of automated dairy product plants. Two of the six contract reports were published last year. The status of the work on the four remaining reports in this series is as follows:

1. Plants Manufacturing Ice Cream and Ice Cream Novelties. At the end of the report year, the contractor's report had been checked, reorganized, and a revised draft for Department publication was about 85 percent completed. The significant results of this study show that an ice cream plant handling 200,000 gallons annually, using automated equipment and an improved layout, could reduce its annual labor costs \$3,250 compared with that for a non-automated plant having a typical layout; a plant handling 1,000,000 gallons of ice cream and 250,000 gallons of novelties annually can reduce its labor costs \$33,500.

2. Plants Manufacturing Cottage Cheese, Cream Cheese, and Cultured Milk and Cream. A report for Department publication prepared from the contractor's report is about 50 percent completed. Results of this study covering layouts and operating criteria for automated and highly mechanized plants manufacturing cottage cheese, cream cheese, and cultured milk and cream show that a plant receiving 172,000 pounds of milk daily and processing it into a product distribution that is 75 percent small-curd cottage cheese, 10 percent cream cheese, 10 percent cultured milk, and 5 percent cultured cream, can reduce labor costs \$75,500 by using automated equipment and an improved layout.

3. Plants Manufacturing Cheddar Cheese. The contractor's report covering layouts and operating criteria for automated and highly mechanized cheddar cheese plants shows that a plant handling 800,000 pounds of milk weekly can reduce its labor force from 43 workers to 22 workers and labor cost from \$279,500 to \$143,000. The use of automated equipment and an improved layout makes it possible to increase labor productivity from 45 pounds of cheddar cheese per man-hour to 88 pounds per man-hour.

4. Sweet Cream Butter and Dried Nonfat Milk. Department work on this contract report has not progressed to the stage where significant findings can be summarized.

5. Consumer Packages and Shipping Containers. A preliminary review of four packaging systems (bottle, can, coated carton, and bag-in-box) reveals that a great variety of milk packages of many different sizes and shapes has been developed, often with little regard for dimension compatibility within or between systems. This is complicated somewhat because bulk packages do not follow the usual practice of marketing milk in a series of binary or half-units. To show how compatible market units and dimensions can improve the marketing of milk, a series of six package models ranging from 1/2-pint to 2 gallons in size has been constructed. Like the bulk bag-in-box containers now in use, milk in this family of packages would permit its storage and handling with a 30 percent saving in refrigerated space required for bottles and cans. The package family offers the additional advantage of a full range of market units in compatible dimensions. A preliminary report is in preparation, describing the family of milk package models, and their possible use in an automated transportation system.

PUBLICATIONS - USDA AND COOPERATIVE PROGRAMS

None.

COOPERATIVE MARKETING Farmer Cooperative Service

Problem: Farmers are expanding their use of cooperative marketing. There are constant changes in transportation, processing, and distribution technology, and in market organization and practices, and changes on the farm itself. In view of these developments, farmer cooperatives and other marketing firms require research results to perform both efficiently and effectively. Such research can assist farmers to maintain and strengthen their bargaining power, increase efficiency, and meet the quality, quantity, and service needs of today's food and fiber marketplace.

Cooperative marketing is a major way for farmers to get maximum returns from their products in the current and rapidly changing market. Farmers own and control cooperatives specifically to increase their income from crops and livestock. Gains are not automatic, however. Cooperatives must plan, develop, and actually manage the specific marketing program and services that will yield the most for their members. Marketing cooperatives must know what the market demands. They must be able to compute the probable cost of different ways of serving the market. They must understand the possibility of major economies in a well coordinated joint sales program, and understand the methods and potentials of bargaining. Management must achieve minimum costs through improved organization, good use of existing plant and personnel, and the selection and use of new equipment and methods.

USDA PROGRAM

The Department conducts a continuing long-range program of basic and applied research and technical assistance on problems of marketing farm products cooperatively. Studies are made on the organization, operation, and role of farmer cooperatives in marketing. While most of the research is done directly with cooperatives, the results are generally of benefit to other marketing firms. The work is centered in Washington, D.C. Many of the studies, however, are done in cooperation with various State experiment stations, extension services, and departments of agriculture.

Federal professional man-years devoted to research in this area totaled 23.3. Of this number, 1.0 was devoted to cooperative marketing of citrus, 2.7 to cotton, 4.5 to dairy, 1.2 to deciduous fruit, 2.2 to grain, 3.9 to livestock and wool, 1.3 to oilseeds and peanuts, 1.0 to potatoes, 3.5 to poultry, 0.1 to rice, 0.6 to tobacco, and 1.3 to vegetables.

Research also is conducted under contract with land-grant colleges, universities, cooperatives, and private research organizations. During the period of this report, contract research was performed by universities and colleges in Florida, Iowa, Louisiana, Montana, North Dakota, and West Virginia, and by one private research company.

STATE EXPERIMENT STATIONS PROGRAM

The State stations maintain a very broad research program in commodity marketing, the findings of which are valuable to cooperatives and to other marketing firms. There are at this time nine projects in eight States that deal specifically with cooperative marketing. Five projects are commodity oriented and deal with grain, tobacco, milk, livestock, and fruits and vegetables. These projects seek to find out how cooperatives are adjusting or might better adjust to changes in market structure and marketing practices. In some instances researchers are studying the success and failure of cooperatives and the organizational structure. One study of the history of major cooperative marketing associations in the State will be published as a book and will undoubtedly receive nationwide attention.

Because of the growing interest in the role of cooperatives in market structure, one State recently initiated a major project in this area. The project leader views cooperative enterprises as a structural dimension of farm markets. The objectives and operating procedures of cooperatives will be studied to see if they have a unique impact upon market conduct and performance. If so, this may have significant implications for Government policies and programs.

The total research effort on cooperative marketing in the eight States is 3.4 professional man-years.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Coordination of marketing

Farmers and their cooperatives need to adapt their marketing methods to the requirements of large-scale buyers, mass merchandising, and other changed conditions. In many cases the coordination of marketing of a number of cooperatives, marketing the produce of hundreds or thousands of farmers, will satisfy these needs and improve returns to farmers. Such coordination may be accomplished by establishment of joint sales agencies or by other methods. Research to determine the problems and needs, and to develop guides for adopting new practices, included work with several commodities.

1. Dairy. Milk is more mobile than before, and there are opportunities to obtain large cost savings through the use of larger milk plants employing new technology. These changes bring a great need for better coordination among cooperatives in milk marketing. A study was made of alternative methods of coordinating the fluid milk marketing of four cooperatives. The study showed that farmers could reduce the cost of packaging and distributing milk about one dollar per hundredweight by using one large modern plant. Economic feasibility of a proposed milk drying plant was studied. It was found that farmers could receive greater returns by coordinating

their marketing through existing cooperative plants rather than by building and operating a new plant. Analysis of six cooperatives showed that coordination of milk marketing through a single sales agency would tend to stabilize market conditions and greatly increase returns to farmers.

B. Improving cooperative sales and distribution methods

In many commodity fields, wholesale and retail marketing practices have changed so much that sales and distribution methods need to be restudied from the farm level forward. Research on these problems included work in several commodities.

Dairy. Research was completed concerning the marketing of nonfat dry milk to domestic commercial outlets. It was found that cooperative manufacturers were able to meet exacting specifications and provide sufficient quantities for commercial users. A need was shown for more information based on research concerning the effect of different types of nonfat dry milk on various finished products. Communication between users and manufacturers needs to be strengthened to insure that users get the best kind of nonfat dry milk for their use, and that plant processing practices employing nonfat dry milk are making the best use of this ingredient.

Staff members are participating in study of the changed market structure for dairy products in the Midwest, by university researchers in that region.

C. Potentials in cooperative marketing

In several commodity areas an appraisal is needed of the present and potential role of cooperative marketing. Current information on cooperative operations can be related to production and marketing conditions. This research will yield suggestions about cooperative operations and services, and provide current data needed by cooperative leaders and others for planning and implementing cooperative marketing programs.

Dairy. A study of cooperative marketing activities and facilities was initiated. This research will include analysis of potential benefits to dairy farmers from increased coordination of their current activities.

D. Pooling and pricing

Pooling principles and procedures must be periodically examined as to their effect on equity among members and efficiency in marketing. In some commodities, pooling has not come into widespread use, and the use of pooling needs to be studied and its application considered.

Dairy. New research was initiated on problems involved in cooperative pooling of expenses and returns in marketing members' milk.

E. Improving operating methods in processing and storage

Studies were underway in several commodity fields to examine new methods, equipment, facilities, and structures for efficient and safe processing and storage of agricultural products by cooperatives.

F. Cost and efficiency

Research studies were undertaken to develop more efficient marketing practices and procedures through analysis of costs involved in using various kinds of facilities and methods of operation.

Dairy. Case studies were made of the organizational structure, operating methods, and costs of large multiproduct cooperatives. The studies showed both advantages and disadvantages in designing a flexible rather than specialized dairy plant.

G. Improving the organization, financing, and management practices of marketing cooperatives

Studies were made to determine way to improve the efficiency and assist cooperatives improve their services by analysis of organization, financing, and management practices.

Dairy. Financing of selected bargaining cooperatives was studied, showing that as marketing services were expanded, the historic service fees have become inadequate. Changes are required in order to maintain present services and also maintain a sound financial structure. A study of member representation in one cooperative developed proposals for more equitable member representation on the board of directors. It was shown that a sound plan for member representation in policy decisions is necessary to maintain sound and generally acceptable policies, particularly following cooperative growth through consolidation and merger. Study of a large and growing manufacturing cooperative showed the need to revise the supervisory control system as a cooperative grows, in order to keep operations efficient, and get maximum returns for farmers.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Davidson, D. R. 1963. Dairy Co-ops Make Impact on Federal Order Markets. News for Farmer Cooperatives (Nov.)

Tucker, G. C. 1964. Dairy Cooperatives Have Year of Decision. News for Farmer Cooperatives (Jan.).

MARKET POTENTIALS FOR NEW PRODUCTS AND USES
Marketing Economics Division, ERS

Problem: Increased emphasis should be placed on new products and new uses because of their importance in expanding markets and maintaining a high rate of economic growth. Agricultural producers and processors need to take maximum advantage of the opportunities offered with respect to additional outlets for surplus supplies, increased returns, lowered costs, and improved competitive positions relative to non-agricultural products. Continuing evaluations are needed of the commercial feasibility and market potentials of new or improved agricultural products, by-products, and products from new crops in food, feed, and industrial uses; of the economic feasibility of developing new uses and establishing new crops, including appraisal of their impact on present markets; and of the economic and technical requirements of end-uses. Such evaluation will provide a sound economic base for decisions on commercial developments, as well as information to guide further utilization research by physical scientists.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving agricultural economists and personnel with dual economic and technical training engaged in research to bridge the gap between laboratory developments and commercial adoption to assist producers to realize more rapidly and more fully benefits of lowered costs, increased returns, and expanded markets that new products and new uses can afford. Research is carried on in industrial and food uses at Washington, D.C., and six field offices -- agricultural economists are located at each of the four Utilization Research and Development Divisions, New Orleans, Louisiana; Albany, California; Philadelphia, Pennsylvania; and Peoria, Illinois. Economists are also stationed at the Hawaii Agricultural Experiment Station, Honolulu, Hawaii, and at the Department of Agricultural Economics, Clemson University, Clemson, South Carolina.

Research is conducted on animal products, cotton, grain and forages, oilseeds, horticultural crops, new crops, and on impacts of technological innovations. Cooperative research is conducted with the Hawaii Agricultural Experiment Station on Kona coffee and Hawaiian fruits and vegetables, with the Pennsylvania Agricultural Experiment Station on maple products, with the Louisiana Agricultural Experiment Station on a new sweetpotato product, and with Clemson University on market potentials for modified milk. Producer groups, such as the Louisiana Sweetpotato Commission and the Michigan Apple Commission, contribute to studies of potentials of new products pertaining to their area of interest.

The Federal scientific effort devoted to research in this area totals about 19.9 man-years. Commodity-wise, 4.7 man-years are currently devoted to animal products; 3.1 to grains; 2.6 to oilseeds and sugar; 3.9 to horticultural crops; and 5.6 to other research, principally new crops and impact of technological innovations.

PROGRAM OF STATE EXPERIMENT STATIONS

Little, if any, research in economics is carried out in this area by State agricultural experiment station personnel. Much research is being conducted on the development of improved products and uses, but it is in the area of technology.

PROGRESS -- USDA AND COOPERATIVE PROGRAM

1. Market Potentials for Low-Fat Milk. Research to measure the market position of low-fat (two-percent) milk and to evaluate its impact on consumption has been completed. Sales of low-fat milk appear to be beneficial to the dairy industry. Although the product displaces whole and skim (other than low-fat) fluid milks to some extent, it has brought new users of fluid milk into the market place. Total fluid milk sales do not appear to be increased by the product's sale. However, its pattern of use indicates that declines have been partially offset in whole milk volume that otherwise might have taken place. Return from milk sales to those consumers who are established users of low-fat milk are greater than they would be if the product were not available.
2. Market Potential for Modified Milk in the Southeast. As a part of research to determine the product mix that will maximize milk consumption, work has been initiated in cooperation with Clemson University to evaluate market possibilities for and impacts of a modified milk. Research to date has dealt with ascertaining the optimum product composition in terms of butter-fat, non-fat, and total solids levels to be used in full scale market testing. After this phase is completed, it is anticipated the product selected will be subjected to vigorous market testing to determine acceptance, sales rates, and impacts on other milk product sales and total sales to provide guides for decisions for the industry.
3. Institutional Market Potentials for Sterile Concentrate. Plans are being developed to undertake, in cooperation with the Wisconsin Agricultural Experiment Station, a research program to evaluate sterilized milk concentrate in institutional markets. The work will be designed to determine the place of such a product among the products the dairy industry offers for sale; i.e., the particular markets and uses where it might be advantageous to sell a sterile concentrate as well as its potentials and impacts.

MERCHANDISING AND PROMOTION PRACTICES
Marketing Economics Division, ERS

Problem: Problems of selling efficiency, consumer acceptance, orderly distribution, and coordination have grown in scope and complexity as major changes have occurred in the production, processing, and distribution of farm products. Because of the wide array of products made available to consumers, through self-service retailing, as well as other factors, merchandising, promotion, the control of product distribution and movement, and management decision-making have increased in importance as basic and essential functions in the marketing of farm products.

Because of self-service not only must a product offered in today's supermarket be its own salesman but also it must compete directly or indirectly with thousands of other items for consumers expenditures. Substantial sums are being spent by farm groups, food processors, and retailers in merchandising and promotional efforts.

Information is needed by producer groups as well as distributors at other levels in the marketing channels to determine the effectiveness of alternative promotion, merchandising and advertising techniques, levels of promotional intensity necessary for maximum sales response and the characteristics of products lending themselves to promotional stimuli. There is a basic need for development of principles or guidelines broadly applicable to agricultural commodities and which may be used in developing and strengthening commodity promotion. More effective merchandising of farm products is also required if demand is to be influenced and greater consumer acceptance gained for farm products. Sales of individual products have become increasingly dependent on in-store merchandising that will attract consumers and influence purchases. This is particularly true for many farm commodities which are not pre-sold through intensive advertising.

Because of increased complexity of operation, firms processing and distributing farm products need information which will assist in improving management efficiency. Smaller firms and particularly those operated by producers often do not have the resources or experience necessary to develop the information or techniques necessary for more efficient operations.

USDA AND COOPERATIVE PROGRAMS

The Department has a continuing long-term program of research in merchandising, management analysis, product distribution, and promotion evaluation to provide information which can be used by producers, handlers and distributors in strengthening and expanding markets for farm products. The merchandising research program is designed to quantitatively evaluate the impact of selected selling practices and price policies on the demand for agricultural products. Specific studies have as their objectives the development

of income-expenditure elasticities and measurement of other factors influencing demand, determination of consumer and market profiles, and evaluation of alternative merchandising techniques such as packaging, display, pricing, featuring and product variation on consumer purchases.

Research relating to promotion and advertising includes studies to: determine organizational structure and procedures of commodity promotion groups for optimum control, coordination and conduct of their program; measure levels of advertising and promotional intensity required to influence sales, evaluate relative effectiveness of alternative promotional appeals, themes, and techniques, and develop principles applicable to the promotion of farm products.

Studies of product distribution, such as availability, movement of products into consumption, and profiles of markets and consumers, provide information by which sound advertising, merchandising and management decisions can be made. In addition, management type studies are conducted to provide techniques and procedures which can be used to coordinate the diverse marketing functions and improve efficiency of firms distributing farm products. Most merchandising and promotion studies are conducted in close cooperation with producer or industry groups, food wholesalers, and retailers. Industry groups giving direct financial support to research during the year include, the American Dairy Association, the Florida Citrus Commission, and Florists' Telegraph Delivery Association.

During fiscal 1964, approximately 14.5 professional man-years were directed to the area of merchandising and promotion. Of this total, 2.0 were devoted to dairy; .3 to beef; .3 to poultry; 1.1 to grains and forage; 3.5 to citrus and subtropical fruits; 1.1 to deciduous fruits and tree nuts; 2.0 to flowers, ornamentals and shade trees; and 5.2 to cross-commodities.

The research effort is centered in Washington, D. C., with professional employees stationed at State Experiment Stations in Washington and Indiana. Cooperative studies are being conducted with the following State Experiment Stations: Arizona, Indiana, Ohio, and Washington. Many studies involve data collection on a national basis while others involve case studies, and controlled experiments in selected locations.

PROGRAM OF STATE EXPERIMENT STATIONS

Much of the research at the State agricultural experiment stations in the area of merchandising and promotion is carried out in connection with specific commodities and thus reported under those headings. That reported here is problem-oriented and only incidentally commodity-oriented. Thus, to get the total effort in this area one would need to add that reported under the specific commodity sections of this report.

Consumer acceptance, preference and attitude studies represent an on-going phase of the State program. Current work deals with food and fiber items

as well as nursery products. This research is undertaken for the purpose of market test and development and, thus, is closely allied with the product development phases of the Stations program. In the year reported, this research totaled 11.4 professional man-years.

Research on consumer motivation and decision-making is underway at 15 State stations. These studies are concerned with type and amount of influence resulting from food promotion and consumer information programs, with factors affecting food purchase decisions, and with consumer behavior in the market place. Limited work is also underway on improved consumer grades for agricultural products. This phase of the market development research totals 17.7 professional man-years.

Research Example--Children's Role in Influencing Food Purchases, Miss. Project 1238.

While children's influence upon family food practices is readily accepted, there is little knowledge about the nature and extent of their role. The Mississippi Agricultural Experiment Station reports that nine- and ten-year-olds are like their elders in that they know somewhat more about food needs than they put into practice. The usual reason for not eating needed foods was that they were not provided by the family. The children studied learned about food from many sources, especially those involving social activity. Market promotion schemes (labels, coupons, and premiums) seemed to have little effect upon them. They reported that requests of parents to buy a food were usually granted, and that all types of food were requested--meat, vegetables, sweets, and soft drinks. This study concluded that the role of the mother in influencing children's food patterns should not be underestimated.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

Effect of Different Levels of Promotion Outlay on Sales of Fluid Milk.

A study was initiated in March 1963 in cooperation with the American Dairy Association to measure the impact of different levels of promotion on sales of fluid milk. Levels of promotional expenditures being tested included 15 and 30 cents per capita above present levels of expenditure on an annual basis. Present levels of expenditures are being used as the control or base. The alternative levels of promotional expenditures are being tested in six major market areas over a 2-year period which will end in February 1965. In addition to sales data, information on levels of employment, school enrollments, and merchandising practices employed in sample retail food stores will be used in evaluating sales of the different levels of promotional expenditure.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

None.

DISTRIBUTION PROGRAMS
Marketing Economics Division, ERS

Problem: For several decades, farming has outrun the manufacturing and distributing industries in achieving greater average production efficiency. An orderly adjustment to this technological revolution has been facilitated by continuing efforts to expand commercial markets and to utilize excess agricultural productive capacity in supplementing diets of children and needy persons. In August 1964, the Food Stamp Program for needy persons was shifted from a pilot effort to a full fledged Program--a part of the overall domestic food aid operation which includes also the National School Lunch, Special Milk, and Commodity Distribution Programs.

There is continuing need for research which will contribute to the attainment of the objectives of distribution programs--constructive use of surplus agricultural resources and improved nutrition for eligible recipients. Primary research relating to consumption, markets, and distribution and research relating to operational effectiveness assist program administrators in the development of new and the modification of existing programs. Findings relating to attainment of basic objectives and the impact of current or alternative distribution programs upon markets for agricultural commodities, prices, and farm income provide guidelines for broader policy determination.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing program of research, basic and applied, which is designed to facilitate the full and effective use of distribution programs in creating an expanded market for agricultural products and improving the national health. The primary research effort is concerned with development of new information through special surveys and the application of findings to the operation of distribution programs. Although projects may be undertaken independently, most involve the joint efforts of agricultural economists, human nutritionists, and program specialists.

The distribution programs research staff has its headquarters in Washington, D. C. This staff conducts surveys throughout the United States and supervises work performed by contractors and other Government agencies. One cooperative project is underway with the Minnesota Agricultural Experiment Station.

The Federal scientific effort devoted to research in this area during the past year totaled 3.8 professional man-years. Of this number, 2.3 was devoted to evaluation of food stamp and direct distribution programs; 1.3 to school lunch and special milk programs; 0.2 to away-from-home eating and other related studies.

PROGRAM OF STATE EXPERIMENT STATIONS

No reports applicable.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

Evaluation of Special Milk Program

A report has been prepared on milk services available to pupils in public and private schools. Preliminary findings indicate that the value of fluid milk consumption, per pupil, increased from about \$8.94 during 1957-58 to \$10.02 during 1962-63. Per capita consumption of other dairy products, including butter, increased moderately from \$1.82 to \$1.98. During the same period, the number of public school children having access to school milk increased by 8 million--from 30 to 38 million children.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

None.

MARKET STRUCTURE, PRACTICES, MARGINS
COSTS, AND EFFICIENCY
Marketing Economics Division, ERS

Problem: The purpose of this research is to find solutions for economic problems in marketing dairy, poultry, and meat animals and their products. More specifically, it is to find answers to the needs of farmers, marketing agencies, and the public for economic knowledge about these commodities--needs for economic knowledge that is relevant to marketing decisions and to the shaping of public policy and programs. This project includes studies of margins, costs and efficiency; of the structures of the systems for marketing individual products; and of the methods and practices followed by farmers, marketing firms, and related public agencies. It provides accurate information about the form and working of the marketing system as a basis for initiating desirable changes and for keeping all parts of the system abreast of technological and economic progress.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program of economic research to assist farmers and marketing agencies to adapt to changes in the environment in which they operate. Work in this area is conducted at Washington, D. C. and in cooperation with State agricultural experiment stations at Durham, N. H., Athens, Ga., St. Paul, Minn., Ames, Iowa, Fort Collins, Colo., Stillwater, Okla., and College Station, Texas. The Federal scientific effort devoted to economic research in this area totals 33.3 professional man-years, distributed as follows: dairy 10.0, swine 0.4, beef 0.5, livestock (cross-commodity) 10.7, and poultry and eggs 11.7. By functional areas, it is distributed as follows: structures, practices and competition 15.0, product quality 3.1, information, outlook and rural development 0.7, and margins, costs and efficiency 14.5.

PROGRAM OF STATE EXPERIMENT STATIONS

All the State experiment stations are conducting economics research dealing with the marketing of animals and animal products.

Dairy marketing research is concerned with the structure, marketing practices and the competitive position of the dairy industry; the effect of promotion and advertising on the sale of milk and dairy products and the margins, costs and efficiencies involved in the assembly, processing and distribution of milk and dairy products. Four regional projects are underway. NCM-26 and WM-46 are investigating structural changes occurring in the dairy industry in response to changes in supply, demand, technology, economic and institutional factors and the effects of these changes on the performance of the

firm and the industry. SM-10 is studying the institutional forces affecting the Grade A milk industry, the supply of milk for manufacturing, the movement of milk to market areas and plant organization and operations. NEM-25 is evaluating economic adjustments in the assembly, processing and distribution of milk in response to changes in labor costs, technological advance and other developments.

The State effort devoted to dairy marketing research amounts to 38.11 professional man years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Structure, Practices and Competition

1. Price Wars in City Milk Markets. Because of the typical structure of the industries and the nature of the competitive process, it is difficult to have the benefits of price competition among milk distributors without it taking the form of price warfare.

Most of the price wars studied had one or more of these aspects which tended to diminish the performance of the industry: (1) Severely depressed prices; (2) destructive competitive tactics; or (3) wasteful forms of innovation.

Dairy farmers supplying markets affected by price warfare among distributors were substantially protected when covered by Federal orders setting minimum producer prices but their ability to negotiate premiums over these minimums was diminished.

The quality of milk was not affected by price warfare except for a tendency to reduce the butterfat content to the legal minimum under local ordinances.

In spite of certain destructive aspects, price competition (even in the form of price warfare) is required for the satisfactory performance of fluid milk industries--to permit innovation of new forms of packaging, new products, or new ways of doing business. Price competition may perform a similar function when new firms seek to enter a market. Perhaps the most important function of price warfare is in compelling a revision of outmoded structures of prices and practices to enable the industry to maintain a satisfactory pace of progress and to improve efficiency.

The study shows that there is ample vitality of competition in city milk industries provided it is neither stifled (by Government or by the firms themselves) nor allowed to degenerate into a continuous state of price warfare. The taking over by Government of the price-making function would not be conducive to workable competition. It might, however, be useful for Government to experiment with hearing procedures to assist in the formation of price structures in those city milk markets which are plagued by chronic price warfare.

2. Structure of the Dairy Industries. New technology, pronounced changes in distribution and merchandising, and other developments have been accompanied by sharp declines in numbers and increases in average size of dairy marketing establishments and firms which may accentuate antisocial behavior in pricing and other trade practices and impair industry performance.

The sharp decline in numbers and increase in average size of producers has been effected partly by a comparatively rapid turnover of producers. Substantial numbers of medium and large fluid milk marketing cooperatives have been involved in consolidations during the past decade. As a result of this movement and the demise of some of the weaker organizations, the total number of such cooperatives has declined considerably. Remaining organizations are generally larger and provide more marketing functions than their counterparts of the mid-1950's.

Sharp declines in numbers and increases in average size have been occurring among most types of dairy marketing establishments. Technological developments which have increased capital requirements and pushed economies of scale to higher levels have contributed importantly to this trend. Because many single-plant firms have gone out of business, and numbers of plants operated by multi-unit firms have increased relatively, if not absolutely, there have been similar, and perhaps more pronounced, trends in numbers and sizes of firms. Nevertheless, considering all industries and markets, the market shares of the national dairies and other dominant firms have by no means universally increased. In the fluid milk industry, for example, widening of distribution areas has helped to maintain the number of milk distributors competing for outlets in a given population center.

Plants manufacturing dairy products decreased by one-third between 1944 and 1961 but the average number of products made increased from 1.5 to 2.0 per plant. Only one product was produced in 44 percent of the plants in 1961, compared with 72 percent of the plants in 1944. In 1961, about 47 percent of the plants had some degree of diversification, compared to only 10 percent in 1944.

Concentration--the share of the market held by the four largest firms--in fluid milk markets has been increasing in recent years in small markets, but it has declined in the largest. Firms packaging fluid milk have been declining in markets of all sizes. The decline in the number of handlers is not a recent phenomena. It has been going on for at least 75 years.

The greatly expanded role of supermarkets and of chainstore organizations in the marketing of dairy products has been a major structural development. Large numbers of food chains now handle private label brands of fluid milk and ice cream as well as evaporated and dry milk and occasionally butter and cheese. Tied in with this development has been a long term increase in the proportion of most manufactured dairy products shipped directly to chainstore warehouses, bypassing old-line wholesalers and jobbers. In the case of butter, this development has had associated with it an increase in the proportion marketed by cooperative sales agencies.

3. Long-Distance Movement of Bulk Milk. A survey of the long-distance movement (over 200 miles) of market milk in the United States showed that:

- (1) In 1960-61 about 424 million pounds of market milk was shipped by over 400 plants mostly in Iowa, California, Virginia, Wisconsin, and Indiana; (2) States receiving most this milk were Texas, Missouri, and Ohio; (3) most shipments were made on a regular basis; (4) nine haulers, operating nearly 200 tank trucks, hauled most of the hauling; and (5) on the average, hauling rates increased about 16 cents per hundredweight per 100 miles over distances ranging from 355 to 1,444 miles. Where backhauls were available, hauling rates were 15 to 20 percent lower.

4. Marketing Milk in Alaska. A comparison of data for four Alaskan markets --Anchorage, Fairbanks, Juneau, and Kodiak--showed that the following changes had occurred since 1957: (1) Total sales of bottled milk (fresh milk, recombined milk, etc.) increased 18.3 percent although sales of fresh milk declined 13.8 percent; (2) total sales of canned liquid and dry milks increased 30 percent; (3) retail prices for fresh milk rose in 3 of the 4 markets; (4) considerable quantities of fresh bulk milk are being shipped to Anchorage at a cost about 10 percent less than local milk; (5) retail price differences between fresh milk and its substitutes have widened. Another important change since 1957 has been the switch by the Department of Defense of serving troops fresh milk rather than recombined milk.

Local Alaskan milk is being displaced from local civilian and military markets by an increasing volume of fresh whole milk shipped in bulk tanks from the West Coast. This is causing an agonizing reappraisal of the future of the dairy industry in Alaska.

5. Management Information Systems. The efficiency of fluid milk marketing firms and the profits of their owners can be materially increased by providing the managers of these firms with information tailored to their needs. A case study in one firm indicates that nearly all of the basic information needed for greatly improved management decisions was already available within the company. It was being used in reports to a variety of regulatory agencies. By putting this information in a form needed by the decision-makers in the company, it provided a basis for greatly increasing the efficiency of the firm.

B. Margins, Costs and Efficiency

1. Costs and Margins of Fluid Milk Distributors. Profits before taxes of fluid milk distributors reached their lowest level in 12 years in 1963. They averaged an estimated 20 cents per hundred pounds of milk and cream processed, about half the level from 1954 to 1961 and about a third that reached during the Korean War. Costs also declined in 1963 but not as rapidly as prices received. These general trends continued into the first quarter of 1964.

2. Efficient Organization of the Southern Dairy Industry. Costs of assembling supplies of fluid milk could be lowered if they were moved more closely to optimum conditions. However, to obtain such conditions would entail considerable reorganization of the assembly patterns in milkshed areas.

The expected patterns of fluid milk distribution at equilibrium in 1975, based on projections of supplies and demand by geographic areas to that period, would be for fluid milk to move generally South from Tennessee and North Carolina, into Alabama, Georgia, and Florida. Some milk would move from the Southern parts of Georgia and Alabama to Florida.

3. Capacity and Flexibility of Milk Manufacturing Plants. Plant milk receipt volumes in the low season of the year represent less than 50 percent of those volumes during the flush period. Milk receipts generally display a relatively long flush period extending from January through June with a peak in March. The low volumes occur quite commonly in September. It is estimated that during the flush season plants are operating between 60 and 90 percent of plant capacity. Plant managers generally see specialization of product as the alternative most feasible and economical for continued operation. Institutional as well as economic factors weigh heavily in the operational decisions of small and medium size plants.

4. Managing Milk Supplies. In the first case study--of a market with an intermediate degree of centralization in management of milk supplies--it was found that greater centralization of management would reduce needed reserves of milk substantially and lower marketing costs. Further case studies--in markets with greater and lesser degrees of centralized management--will serve to pinpoint the costs of decentralized management and savings which are possible.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

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- Klein, J. E., and Gray, L. R. December 1963. Drive-in dairies in central California. Development, organization, and operation. MRR-636. 11 pp.
- Manchester, A. C., and Sitzman, L. Rev. April 1964. Market shares in fluid milk markets. 73 pp.
- Reichert, C. F. March 1962 (released 1964). Market organization, promotional activities, and practices of fluid milk plants in the Western region. Oreg. Agri. Expt. Sta. Tech. Bul. 59, Western Reg. Pub. 52 pp.
- Russell, S. July 1964. Producer delivery patterns in New England markets. MRR-672. 111 pp.
- Wolf, A. F., and Sitzman, L. Quarterly. Milk distributors' sales and costs. 6 pp.

CONSUMER PREFERENCE AND QUALITY DISCRIMINATION--
HOUSEHOLD AND INDUSTRIAL
Standards and Research Division, SRS

Problem. With the increasing complexity of marketing channels and methods, it has become almost impossible for consumers to express to producers either pleasure or displeasure with available merchandise. To market agricultural products more effectively, it is necessary to understand existing household, institutional, and industrial markets and the reasons behind consumers' decisions to purchase or not to purchase. Information is needed on consumers' attitudes toward old and new product forms of agricultural commodities, preferences, levels of information or misinformation, satisfactions or dislikes, and what product characteristics would better satisfy current consumers and/or attract new ones. It is also important to know the relationship between the consumption of one agricultural commodity and another in consumers' patterns of use, the relationship between agricultural and nonagricultural products, and probable trends in the consumption of farm products. Producer and industry groups as well as marketing agencies consider such information essential in planning programs to maintain and expand markets for agricultural commodities which, in turn, increases returns to growers.

USDA PROGRAM

The Special Surveys Branch conducts applied research among representative samples of industrial, institutional, or household consumers and potential consumers. Such research may be conducted to determine preferences, opinions, buying practices, and use habits with respect to various agricultural commodities; the role of competitive products; acceptance of new or improved products; and consumers' ability to discriminate among selected attributes of a product or levels of an attribute, and the preferences associated with discriminable forms.

In addition to the studies of consumer preference and discrimination, the Branch also provides consultants and conducts special studies, upon request, for other agencies in the USDA or within the Federal Government, when survey methods can be usefully applied to the evaluation of programs, services, or regulatory procedures of interest to the requesting agencies.

The research is carried out in cooperation with other USDA or federal agencies, State experiment stations, departments of agriculture, and land grant colleges, and agricultural producer, processor, and distributor groups. Closely supervised contracts with private research firms are used for nationwide surveys; studies in selected areas are usually conducted by the Washington staff with the assistance of locally recruited personnel.

The Branch maintains all of its research scientists, who are trained in social psychology or other social sciences, in Washington, D. C., which is headquarters for all the research whether it is conducted under contract or directly by the Branch. The Federal scientific effort devoted to research in this area during the past year totaled 7.0 professional man-years. An additional .2 professional man-year was devoted to research conducted under a transfer of funds arrangement.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

Consumer Preference

Milk. A final report is in preparation on a study to ascertain household consumption patterns for fluid milk and to provide insights into the reasons for consumers' use of selected fluid milk products. This survey is part of a research project conducted in cooperation with the Economic Research Service. Since major interest focused on the market potential for low-fat fluid milk of approximately two percent butterfat content, interviewing was conducted in two cities in which low-fat fluid milk was available. Information was obtained on such topics as consumers' attitudes toward various types of milk, milk consumption habits during the preceding five years, changes in those habits, and the reasons for the changes.

COMMODITY SITUATION AND OUTLOOK ANALYSIS
Economic and Statistical Analysis Division, ERS

PROBLEM

Because of the instability of the prices he receives and rapidly changing conditions of agricultural production, the farmer stands in special need of frequent accurate appraisals of his economic prospects if he is to plan and carry out his production and marketing activities in an efficient and profitable way. The typical farmer cannot afford to collect and analyze all the statistical and economic information necessary for making sound production and marketing decisions. It is a goal of the Department to provide the farmer with economic facts and interpretations comparable to those available to business and industry. This is accomplished through a continuous flow of current outlook information, the development of longer range projections of the economic prospects for agricultural commodities, and analyses of the economic implications of existing and proposed programs affecting farm commodities.

USDA AND COOPERATIVE PROGRAM

The program includes the regular publication of 12 commodity outlook reports; holding of the Annual Outlook Conference in Washington in mid-November; participation of commodity specialists at regional and State outlook meetings and at meetings of farm organizations and agricultural industry groups; preparation and publication of special articles bearing on both the short-run and long-run outlook for farm commodities; issuance of comprehensive statistical bulletins containing the principal economic series pertaining to the various commodities; long-range projections of supply of and demand for the major agricultural commodities; and continuing analysis of the impact of existing and proposed alternative farm programs as they affect output, utilization, and prices of these commodities.

Except for a Regional Field Office for Livestock, in Denver, Colorado, all the USDA situation and outlook work is carried on in Washington. The regional livestock project is a cooperative effort including the Economic and Statistical Analysis Division, the Federal Extension Service, and State Extension Services in the Western and certain Great Plains States.

The total USDA commodity situation and outlook program currently involves 21.5 professional man-years.

Dairy. This work involves 2.0 professional man-years in Washington. The outlook and situation program provides a continuing appraisal of the current and prospective economic situation of milk and milk products. Appraisals are published 5 times a year in the Dairy Situation, and quarterly in the Demand and Price Situation and the National Food Situation. A comprehensive analysis of the dairy situation is presented at the Annual Outlook Conference. Outlook appraisals are frequently presented at regional or State outlook meetings, at meetings of farm organizations, and to various agricultural industry groups. Special analyses are made on the probable effect of proposed programs on the production-utilization balance of milk and milk products. Basic statistical series are maintained, improved, and published for general uses in statistical and economic analysis. A supplement to Dairy Statistics is being prepared for release in 1965.

PROGRAM OF STATE EXPERIMENT STATIONS

For the most part the States depend upon the U.S. Department of Agriculture for the yearly across-the-board commodity situation and outlook research. The State extension staff members supplement and adapt such research information to meet the commodity situation of their States.

Four States have projects that deal specifically with analysis of current price trends and prediction of future prices. There is increasing interest in longer range price prediction because of the growing specialization of farms, which make yearly enterprise shifts less common and less feasible, and which calls for large capital commitments over longer periods of time.

The total direct research effort in the situation and outlook area is approximately 1.7 professional man-years. While not designated as outlook research, much of the research conducted by the experiment stations and reported elsewhere contributes to improved understanding of price-making forces, which in turn improves market situation analysis and price forecasting.

PROGRESS--USDA AND COOPERATIVE PROGRAMS

Dairy

In addition to regular analytical and outlook work, considerable effort was devoted to analyzing the effects of existing and proposed Government programs on milk production, marketings, consumption, stocks, and farm income from sales of dairy products. Other work in this area included analyses of changes in imports and exports of dairy products; the Canadian dairy program and its effect on Canadian consumption; and U.S. domestic food distribution programs. The series on milk equivalent of manufactured dairy products was revised, starting with January 1958, to include all products for which monthly data are currently available, and to make account of changes in milkfat content each month. Supplement for 1962 to Dairy Statistics Statistical Bulletin No. 303, was revised in December 1963.

In preparation for a revision of Agricultural Handbook No. 62, Food Consumption in the U.S., tables on supply and distribution of dairy products were revised, and export information for 1955 to date was broken into commercial and uncommercial exports. Work has been started on a revision of ERS-63, Government's Role in Pricing Fluid Milk in the United States, to be published next spring.

The Payment-In-Kind program was analyzed to determine its effect on 1964-65 U.S. supplies of nonfat dry milk and butter, domestic prices, and the availability of these products for foreign markets and donations. The world dairy situation was studied to establish causes of current tight world supplies, and an article on this was prepared for Foreign Agriculture.

A special article was prepared on the outlook for dairying five years ahead. Prospects are that consumption of milk in all forms may decline to about 600 pounds per person over the next five years, down about 40 pounds from 1963. Shifts in consumers' preferences are expected further to favor the solids-not-fat component of milk, a challenge calling for responsive adjustments in dairy production and price structures. If present trends continue, cow numbers will continue to decline, and the number of dairy herds will drop, particularly the number of small herds. By 1968 more than half of the cows will be in herds of 30 or more cows. Production per cow is likely to increase enough to offset the decline in numbers, at least maintaining current production levels. About two billion pounds less milk is likely to be used on farms, thus increasing the supply available for sale.

PUBLICATIONS--USDA AND COOPERATIVE PROGRAMS

Dairy

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